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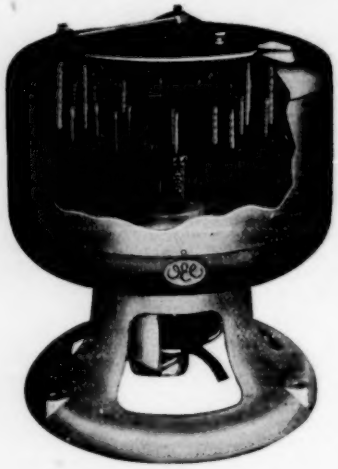

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A STUDY OF THE CHEMICAL AND PHYSICAL PROPERTIES OF REMADE MILK[1]

L. S. PALMER AND C. D. DAHLE

Divisions of Biochemistry and Dairy Husbandry, University of Minnesota, St. Paul, Minnesota

The rapid increase in the development of the powdered milk industry during recent years and the increasing consumption of this product in liquid form has raised several important questions of interest both to the manufacturer of the powdered milk, and to the consumer who buys it, either in reconstituted or reconstructed form, possibly as "fresh" milk. The dairy control laboratories of our large cities whose duty it is to protect the milk supply of their respective communities are also interested in the advent of powdered milk for use in the standardization of market milk or its sale as whole milk after reconstitution from whole milk powder, or its reconstruction from skim milk powder and butter.

Among the important questions raised in this new field of the dairy industry is the question whether powdered milk exhibits the same chemical and physical properties after remaking it into fluid milk as did the original milk from which the powder was produced. Other important questions have to do with the nutritional, especially the vitamine, efficiency of remade milk, and also its safety from a bacteriological point of view.

It is not the purpose of this paper to present data on the latter two aspects of the problem. It is desired, however, to report a study of certain of the chemical and physical properties of milk either (1) reconstituted from whole or partially skimmed

[1] Published with the approval of the Director as Paper No. 286, Journal Series, Minnesota Agricultural Experiment Station. The data presented in this paper form a part of the thesis of C. D. Dahle, submitted in partial fulfillment of the requirements for the degree of Master of Science in the Graduate School of the University of Minnesota, 1921.

milk powders or (2) reconstructed from skim milk powder and butter. In undertaking this study it was not our purpose to try to find a test for remade milk, but merely to examine its properties from a general point of view. In so doing, it was recognized at the outset that there are essentially only three types of powdered milk on the market at the present time. One type has been dried at atmospheric pressure in a thin film on a slowly revolving heated drum according to the basic patents now known as the Just-Hatmaker process. Another, more recent type, has been dried by spraying the previously condensed milk under hydraulic pressure into an essentially closed chamber through which a stream of heated air is continually passing. This process is exemplified by the milk manufactured by the Merrill-Soule process. A third type differs from the Merrill-Soule process in that the milk which may or may not be previously condensed, is sprayed by centrifugal force into a tower through which heated air is being circulated in a cyclonic or some similar manner. Patents controlled by the California Central Creameries Company of Los Angeles, California, and the International Dry Milk Company of Minneapolis, Minnesota, illustrate this type of process.

EXPERIMENTAL

The experiments reported below include an examination of milk remade from each of the three types of process of manufacture and also of milk reconstructed from skim milk powder, water, and sweet, unsalted butter. These milks will hereafter be referred to as drum process, pressure spray process, centrifugal spray process and reconstructed milks, respectively. Fresh powder was obtained at the beginning of the study. From time to time fresh samples made by the centrifugal spray process were obtained. The stock of pressure spray powder was not replenished but new cans from the original lot were opened from time to time. The same lot of skim milk powder was used throughout the experiments in making the reconstructed milk. The drum process powder was renewed once during the course of the study.

Method of remaking the milk. An ordinary egg beater and Dazey churn were used in remaking the milks from whole milk or partly skimmed milk powder and water. Usually just enough milk was made up at one time for the experiment to be conducted. Tap water was used at a temperature of 60–65°C. except in the experiments on freezing point, specific conductivity and buffer value, where distilled water was used. The samples were allowed to cool before using.

A De Laval emulsor no. 2 was used in reconstructing the milk from skim milk powder, sweet butter and water, following the directions put out by the manufacturers of the machine.

PHYSICAL PROPERTIES OF REMADE MILK

Freezing point. The freezing point is one of the least variable of the physical properties of milk. It has been long recognized as the most sensitive test available for the detection of added water in milk, as has been recently confirmed anew by Hortvet (1).

The purpose of examining this property of remade milk was to determine how closely the reconstituted and reconstructed products resemble fresh milk when made up according to the directions given by the manufacturers. In case divergencies occurred it was planned to determine, if possible, the correct proportions of powder and water to use to obtain a product showing the normal freezing point of fresh milk. It was believed that such data would be of value to those making reconstituted or reconstructed milk as an aid in obtaining a product which would resemble most closely fresh cow's milk. It was planned, also, to use the data thus secured in our own subsequent experiments in remaking the milk for the examination of other properties.

Evenson (2) has recently determined the freezing point of milk remade from various kinds of powder. The exact proportions used by him in reforming the fluid milk are not stated. Some variations from the accepted average normal of −0.55°C. were found, but the conclusion drawn was that the differences

are not great enough to make the freezing point of value in detecting mixtures of natural and remade milk, which Evenson was seeking to do.

In our experiments the pressure and centrifugal spray whole milk powders were made up in the proportion of 1 part by weight of powder to 7 parts by weight of water, yielding milk with approximately 12.5 per cent total solids. In the case of the drum process powder, made from partly skimmed milk, the directions of the manufacturer call for mixing 1 part by weight of powder and 8 parts by weight of water, yielding a product containing approximately 11 per cent total solids. In the case of the reconstructed milk 2.29 pounds of skim milk powder were dissolved in 21.67 pounds of water and emulsified with 1.04 pounds of sweet cream butter, forming 25 pounds of milk testing 3.5 per cent fat.

The freezing point was determined by means of the newly designed Hortvet (1) cryoscope, the thermometer having previously been standardized, using solutions of pure sucrose.

The results of these experiments are given in table 1, which shows the freezing point depression as obtained by using the proportions of ingredients recommended by the manufacturers and also the proportions which it was found necessary to employ to secure a normal freezing point.

The data in table 1 show that the manufacturers of the drum process, partly skimmed milk powder, recommend slightly more powder than necessary to give a milk showing a normal freezing point, while the manufacturers of the centrifugal spray process powder recommend slightly less powder than is needed to secure a normal result. It is possible that the latter result is due to the fact that the heat employed in the process denatures slightly the hydrophyllic colloids of the milk so that they do not bind back the water which they naturally contained, the effect being that the milk has been slightly watered. Other factors which may be involved are (1) a high moisture content of the powder, (2) a lower total solid content of the original milk than the standard adopted by the manufacturer.

Specific gravity. The specific gravity of the various kinds of remade milk was determined at 60°F. two hours after being made up according to the proportions giving a normal freezing point. The figures obtained were as follows: drum process milk, 1.0336; pressure spray milk, 1.032; centrifugal spray milk, 1.0314; reconstructed milk, 1.0314. The figures are seen to lie within the limits of variation of normal milk.

Viscosity. It is generally recognized at the present time that the viscosity or fluidity of milk is determined largely by the constituents of milk which are in colloidal solution, namely, the calcium caseinate, the albumin and globulin, and the colloidal

TABLE 1

Freezing point of remade milk

KIND OF MILK	PROPORTIONS RECOMMENDED BY MANUFACTURERS			Δ	PROPORTIONS FOUND NECESSARY FOR NORMAL			Δ
	Powder	Water	Butter, 84 per cent fat		Powder	Water	Butter, 84 per cent fat	
	per cent	*per cent*	*per cent*	*°C.*	*per cent*	*per cent*	*per cent*	*°C.*
Normal cow's milk.....				−0.555				−0.555
Drum.....	11.1	88.9		−0.655	10.6	89.4		−0.550
Pressure spray.....	12.5	87.5		−0.545	12.5	87.5		−0.545
Centrifugal spray.....	12.5	87.5		−0.500	12.8	87.2		−0.548
Reconstructed.....	9.16	86.68	4.16	−0.560	9.16	86.7	4.16	−0.560

di-calcium phosphate, rather than by the fat which is present in particles whose size is considerably above the colloidal realm. Any process of powdering milk which has a profound effect on the colloids of the milk would therefore be expected to manifest itself in the viscosity or fluidity of the remade product.

In determining this property the milks were remade in the proportions giving a normal freezing pont and the observations made at 25°C., while the milk was fresh, and again after standing for 24 hours at 7°C., the reading again being taken at 25°C.

A McMichael viscosimeter was used but the calibrated disc was rotated as in the Doolittle (3) or Mojonnier method. The average of four readings was calculated in terms of centipoises

or absolute units using tables which had been worked out for the instrument in this laboratory.

The results are shown in table 2, in comparison with readings made on freshly pasteurized milk. The data indicate a slight alteration of the colloids in all cases. This is least noticeable in the case of the pressure spray milk and most evident for the drum process milk. The decline in viscosity on standing in the case of the drum process milk is to be explained on the grounds of a greater dispersion of the colloidal material on standing. The increase occurring in all the other cases, including the natural milk, is presumably a hydration effect in the realm of greatest colloidality.

TABLE 2

Viscosity of remade milk

KIND OF MILK	TEMPERATURE	VISCOSITY WHEN FRESH	VISCOSITY AFTER 24 HOURS AT 7°C.
	°C.	*centipoise*	*centipoise*
Pasteurized	25	1.39	3.25
Drum process	25	7.36	5.91
Pressure spray	25	1.79	3.64
Centrifugal spray	25	2.25	4.10
Reconstructed	25	1.94	2.29

Specific conductivity. The specific conductivity was determined in a Freas cell at 25°C. The constant of this cell was found to be 0.34202. The data obtained are given in table 3. With the exception of the drum process milk practically the same values were obtained for all the milks, including the normal milk samples used as a check. The high conductivity of the drum process milk may be due in part to the partial skimming, Durand and Stevenson (4) having shown the skim milk has a higher conductivity than whole milk.

Creaming ability. The amount of cream which rises on milk is used by the public to judge the richness of the milk, notwithstanding the fact that this is frequently an erroneous standard. Remade milk for the most part shows little or no rise of fat to form a cream layer. In the case of milk remade from powder

manufactured by the pressure spray process no cream rises at all because the fat is thoroughly homogenized in powdering the milk. The milk remade from the drum process powder gave a small cream line on standing, the volume of cream amounting to about 2.5 per cent. It will be recalled that this powder is made from partly skimmed milk. Milk which was reconstructed from skim milk powder and butter also threw up a small cream layer (about 3 per cent), but the cream was very rich, almost like butter. It consisted of large globules very closely packed together. Only in the case of the centrifugal spray powder was anything like a normal cream line obtained. This milk, after remaking, showed a cream layer of about 13 per cent, which contrasts very favorably with the usual cream layer of 14 to

TABLE 3

Specific conductivity of remade milk

KIND OF MILK	NUMBER OF DETERMINATIONS	AVERAGE SPECIFIC CONDUCTIVITY
		mhos
Fresh, pasteurized	4	0.00547
Drum process	8	0.00625
Pressure spray	8	0.00545
Centrifugal spray	12	0.00560
Reconstructed	8	0.00570

16 per cent which is obtained for the average pasteurized market milk. The milk in all these experiments, was allowed to cream at 10°C.

General physical appearance on standing. In addition to the cream layer other features of the remade milks were noted. The milks remade from the pressure spray powder and reconstructed from pressure spray skim milk powder and butter exhibited no abnormalities. The milk remade from the centrifugal spray powder, however, showed a large volume of foam-like material on the surface which consisted of floating, partly dissolved granules of powder. The dispersed fat in this milk was very prone to churn when the milk was shaken. It was, in fact, found necessary to exert care in remaking the milk to avoid this churning of the fat.

In the case of the milk made from the drum process powder there was always a separation of solids-not-fat after standing over night. The fluid at this time exhibited 3 layers, a layer of casein-like material at the bottom, a layer of clear serum above this, and a layer of cream on the surface.

CHEMICAL PROPERTIES OF REMADE MILK

Soluble proteins. The so-called soluble proteins of milk, the albumin and globulin, which in reality are present in a colloidal state, begin to coagulate at 72°C. Although even long continued boiling fails to remove all of these two proteins from milk, as one of us (5) has recently shown, nevertheless the content of soluble protein in remade milks should give roughly an index of the maximum temperature employed during the drying process. The analysis of remade milks for total soluble protein should at least indicate whether the powder ever attained a temperature of 72°C. during the process.

The method employed in our experiments was to remove the casein from 10 cc. portions of the remade milk by precipitation with acetic acid, following the usual A. O. A. C. method. The casein was washed, however, with acetic acid water adjusted to a pH of 4.8. The filtrate and washings were neutralized to a faint pink with NaOH solution, using phenolphthalein as indicator and the soluble proteins precipitated with 50 cc. of Almen's tannic acid reagent. After standing for one hour the precipitate was filtered off, washed, and the nitrogen determined in the precipitate by the usual Kjeldahl method.

The results obtained are shown in table 4. The data show that the soluble proteins have been materially lessened during each of the processes except the centrifugal spray, indicating that the heat in these cases must have reached 72°C. The drum process powder obviously attains the highest temperature and the centrifugal spray powder the lowest. The partial coagulation of the soluble proteins in the case of the pressure spray powder probably occurs either during the preliminary condensing process or while the powder is subjected to the continued blast of hot air as it lies in the powdering chamber during a "run."

Rennet coagulability. The possibility of using remade milk for cheese manufacture is worthy of consideration. To be useful for this purpose, however, the remade milk should at least possess the rennet coagulability of pasteurized milk.

In our tests the Marshall rennet cup was used to determine the speed of coagulation and the character of the curd. Several tests were run on each milk with uniform results. Milk made from the centrifugal spray powder was found to resemble closely pasteurized milk both as to the speed of coagulation and character of clot. The pressure spray milk ranked next, a little more time being required to stop the flow of milk from the bottom of the Marshall cup, and the curd being less firm. The drum process

TABLE 4

Soluble protein content of remade milk

KIND OF MILK	AMOUNT N OBTAINED	SOLUBLE PROTEIN (N × 6.38)
	mgm. per 10 cc.	*per cent*
Normal	10 to 12	0.64 to 0.77
Centrifugal spray	11.05	0.70
Pressure spray	8.82	0.56
Reconstructed	8.17	0.52
Drum	7.22	0.46

milk and the reconstructed milk failed to clot, a very fine coagulation of the casein occurring instead.

The failure of the drum process milk to clot normally is in line with the observations of others (2). The reason for the failure of the reconstructed milk to clot probably lies in the repasteurization of the milk during the process of remaking. This point, however, needs further investigation.

HYDROGEN ION CONCENTRATION AND BUFFER VALUE

Evenson (2), has determined the hydrogen ion concentration of remade milks and also their buffer value as compared with pasteurized milk. No differences were noted by him except in a case where the milk had obviously been treated with alkali. He has not reported his results in detail.

We also performed essentially the same experiments with the types of remade milk under investigation in comparison with freshly pasteurized milk. In determining the buffer value of the milks towards both acid and alkali 25 cc. portions of the milk were treated with increasing amounts of 0.1 N lactic acid and lime water, respectively, and the hydrogen ion concentration of the mixture determined electrometrically. The data are presented in table 5. The portion of these data near the initial value are shown graphically in chart 1, after calculating the concentration of 0.1N acid and alkali, respectively, in the samples tested for hydrogen ion concentration.

TABLE 5

Buffer value of remade milk

AMOUNT OF ACID AND ALKALI ADDED TO 25 CC.	PASTEURIZED		DRUM		PRESSURE SPRAY		CENTRIFUGAL SPRAY		RECONSTRUCTED	
	Acid	Alkali	Acid	Alkali	Acid	Alkali	Acid	Alkali	Acid	Alkali
cc.	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.0	6.514	6.514	6.605	6.605	6.607	6.607	6.619	6.619	6.621	6.621
0.1	6.512	6.516	6.579	6.634	6.596	6.629	6.600	6.620	6.549	6.629
0.2	6.499	6.543	6.529	6.637	6.561	6.644	6.526	6.636	6.472	6.650
0.4	6.444	6.610	6.475	6.678	6.494	6.654	6.395	6.695	6.418	6.657
0.8	6.357	6.651	6.371	6.712	6.376	6.712	6.289	6.768	6.285	6.690
1.6	6.147	6.717	6.120	6.765	6.161	6.730	6.093	6.852	6.102	6.850
3.2	5.811	6.854	5.785	6.933	5.750	6.951	5.806	6.993	5.730	6.989
6.4	5.328	7.132	5.285	7.271	5.292	7.295	5.323	7.359	5.297	7.380
9.6	4.923	7.514	4.918	7.743	4.794	7.817	4.850	7.767	4.878	7.940
12.8	4.466	8.021	4.362	8.483	4.386	8.715	4.389	8.747	4.494	8.699

Although the differences are not marked they are uniform in showing that the remade milks all showed less buffer effects at low concentration of acid and alkali than were obtained for the natural milk.

Enzyme activity. The remade milks were tested for peroxidase both by the Storch test, using hydrogen peroxide and paraphenylenediamine, and by the guaiac test using hydrogen peroxide and a tincture of gum guaiac. Except for the drum process milk all the samples gave tests which could not be distinguished from raw or pasteurized milk. Both tests were negative for the drum process milk.

Inasmuch as the peroxidase enzyme of milk is destroyed at a temperature of 78° to 82°C., it is obvious that the powders

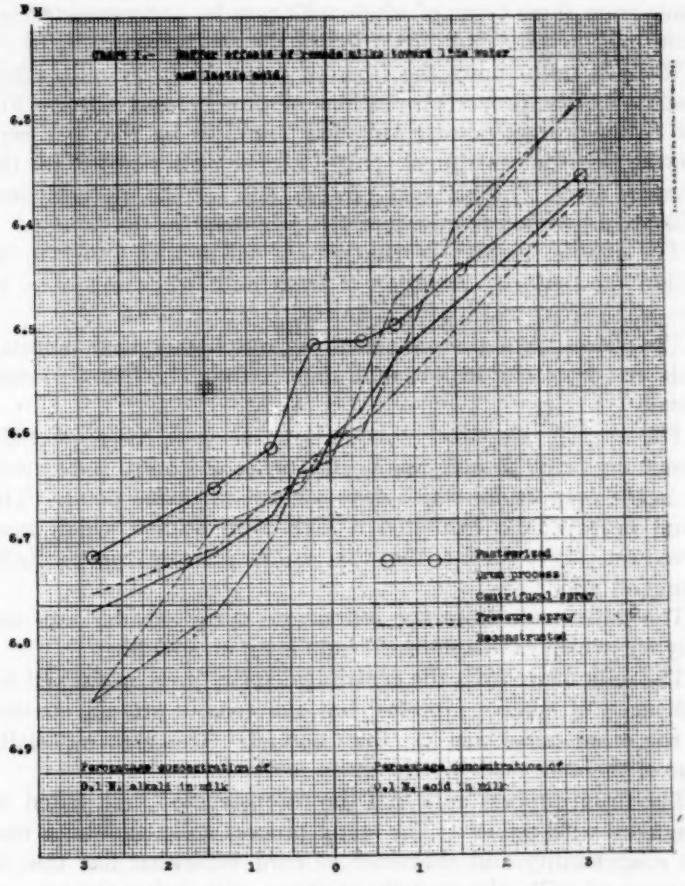

CHART 1

manufactured by the spray process do not attain this temperature while that made by the drum process exceeds this temperature during the manufacture.

SUMMARY

A study of the physical and chemical properties of milk remade from three types of whole milk powder and reconstructed skim milk powder and butter led to the following results:

Remade milk shows the freezing point of fresh milk when made with the proper proportions of powder and water. For the drum process powder this was found to be 10.6 per cent powder, for the centrifugal spray 12.8 per cent powder, for the pressure spray 12.5 per cent powder, and for the reconstructed milk, 9.16 per cent powder and 4.16 per cent butter.

The specific gravity of the remade milks was found to lie within the limits of variation of fresh milk when made up in the proportions which give a normal freezing point.

The viscosity of the remade milks was higher than normal. This was especially true of milk remade from the drum process powder.

The specific electrical conductivity of each type of milk except the drum process was normal when the milks were made up in the proportions which gave a normal freezing point. The drum process milk exhibited a high conductivity which may have been due to the fact that this powder is made from partially skimmed milk.

The milk made from the centrifugal spray powder was the only one which gave a normal cream layer on standing.

The milk made from the centrifugal spray powder showed no deficiency of soluble proteins, but the soluble protein content of the other milks was less than normal. This was especially true of the milk made by the drum method.

The drum process milk and the reconstructed milk failed to coagulate with rennet. The spray process milks showed a rennet coagulability and character of curd somewhat like that of pasteurized milk, the centrifugal spray milk being the nearest to the pasteurized milk in this regard.

At a low concentration of added acid and alkali all the remade milks showed less buffer than normal milk in terms of pH.

Peroxidase was absent from the drum process milk, but was strongly active for the other remade milks examined.

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THE DETERMINATION OF YEASTS AND OIDIA IN CREAM AND BUTTER

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Among the various factors that determine the keeping quality of butter, yeasts and Oidium lactis have been considered of importance by many authorities. Jensen (1902) found that pure cultures of Oidum lactis produced acid by splitting butter-fat in butter, and that this acid caused rancidity. Rogers (1904) reports the fat-splitting enzymes of certain torulae as being responsible in part for the development of acidity in stored butter and suggests that it is reasonable to suppose that they help to produce the so-called "fishy" flavor in butter packed in large vessels. Sayer, Rahn, and Farrand (1909) claim that Oidium lactis, because of its fat-splitting qualities, might be one of the causes of rancidity, but no conclusive proof is offered. Thom and Shaw discuss molds as present and offer suggestions for their control. Combs and Eckles (1917), working with both sweet and sour cream, show that Oidium lactis, as well as Penicillium chrysogenum, when grown in cream previous to pasteurization, exerted a decidedly detrimental effect on the keeping qualities of the butter. This was due to the enzymes carried over into the butter and not to the growth of the mold itself. Eckles (1919) recommends pasteurization at 175° F. to destroy these enzymes and thus insure better keeping quality. Lund (1919) states that butter containing large numbers of yeasts may score high when fresh, but after four months in storage the score will be lower than that of butter containing fewer yeasts. Nicholls (1919) claims that Odium lactis when present is an important factor in the deterioration of butter, and that its enzymes are destroyed only at temperatures above 180°F.

In undertaking to study the presence and function of any group of organisms in butter-making it must be recognized that

many factors may enter into the changes occurring in a lot of cream before churning as well as into the finished butter. Further, it is practically impossible to conduct experiments under conditions which will limit the agencies present to single factors. The results of experiment are, therefore, aggregate effects of several factors rather than evolutions of single factors. Many bacteria survive and multiply in close association with yeasts and Oidium lactis. Studies of the presence and effects of particular species must seek to correlate the presence or absence of the particular organism with the characteristic qualities of the butter in series of experiments rather than in isolated tests.

During the summer of 1918, a series of samples of cream and butter were obtained and examined in Denver. The cream used represented the daily receipts of a large creamery, drawing much of its supply from long distances. No attempt to obtain the complete history of each sample was made. The samples were subjected to extensive chemical and bacteriological study for the general purpose of correlating the condition and quality of the cream used with the characteristics determinable in the finished butter. After many experiments the following adaptation of the Breed[1] method for the microscopic count of microorganisms in dairy products was developed.

Cream. For making microscopic slides, the cream is heated to 30°C. and thoroughly mixed. A Breed pipette, calibrated to deliver 0.01 cc. of cream of the average consistency, is used to transfer the sample to the slide, upon which it is spread over 1, 2, or 4 square centimeters according to the quality of the cream under investigation. As much distilled water as necessary is added when spreading over 2 or 4 square centimeters. The method of spreading over a large area as equivalent to dilution is adopted as requiring less time and being more accurate.

Butter. By means of a small butter trier, 3 or 4 cores from different portions of the butter sample are transferred to a 6 by 1 inch test tube, usually about half filling the tube. The butter is melted at 45°C. and allowed to stand at temperatures

[1] Standard Methods for the Bacteriological Examination of Milk, 3d edition, Boston, 1921, pp. 14–16. Published by the American Public Health Association.

between 45° and 40°C. until the fat is separated in a clean layer with mixture of curd and whey or brine at the bottom of the tube. With a sterile 1 cc. pipette the curd and whey are thoroughly mixed by drawing the liquid back and forth in the pipette. Then 1 cc. of this mixture is transferred to a clean watch glass, care being taken to wipe thoroughly all fat from the outside of the pipette with a clean towel before discharging the pipette into the watch glass.[2] With a Breed pipette 0.01 cc. of the mixture is transferred to a microscopic slide, carefully spread over an area of 1 sq. cm., 2 sq. cm., or 4 sq. cm., depending on the quality of the butter, diluting if necessary with distilled water in order to obtain uniform smears. After drying in the air, extracting with xylol, and fixing with 95 per cent alcohol, the smears are stained with saturated aqueous methylene blue, after which they are washed with water and then with 95 per cent alcohol and dried in the air.

Counting is done with a combination of lens and draw tube length to give a factor of 500,000.[3] (See Standard Methods for the Bacteriological Examination of Milk, 3d edition, 1921, pp. 14–16). In enumerating yeasts and oidia each cell, whether separate or as an element in a chain or budding colony, was counted. The number of fields to be counted will depend upon the number of organisms present. It is recommended that 100 fields be counted except in very high count products.

CULTURAL EXAMINATION

Cream was warmed to 30°C., thoroughly mixed by agitation before portions were withdrawn for examination. In the case of butter, four cores were withdrawn from the sample with a sterile trier and placed in a sterile bottle, warmed sufficiently to make the butter soft and mixed thoroughly with a sterile spatula; 10.1 grams of butter were weighed into a sterile porcelain mortar and enough sterile sand was added to give a dry granular mixture when ground up with a sterile pestle. This was then scraped with a sterile spatula into a sterile 500 cc. wide mouth, glass-

[2] A Petri dish may be used but is not as satisfactory.

[3] The diameter of the field should be 0.16 mm. to give a factor of 500,000.

stoppered bottle, 90 cc. of sterile water was added and the bottle was shaken vigorously until a uniform mixture was obtained. From this 1:10 dilution the higher dilutions were prepared.

In examining the colonies on plates, if there was any doubt as to whether these colonies were of bacteria, yeasts or molds, a water suspension was in all cases examined under the microscope. It was found helpful to have a little methylene blue present in the water used for making these suspensions.

In the cultural examination of the samples, the following data were recorded: Counts of yeasts and oidia by the microscopic method, counts of yeasts and oidia in wort agar and in whey agar after five days at 30°C., a microscopic count of total bacteria by the Breed method, a total count of bacteria upon whey agar after five days at 30°C., a total count on wort agar after five days at 30°C., and determinations of the percentages in the following groups: (1) acid organisms; (2) acid coagulating organisms; (3) alkaline organisms; (4) organisms producing no change; (5) peptonizing organisms and (6) organisms which both coagulate and peptonize. The results of microscopic and cultural examination were brought together, and tabulated.

Careful consideration of the tabulated totals obtained from bacteriological examination of freshly made butter indicated that the actual numbers of bacteria present had little significance. The addition of starter introduced so large a factor of the purely acid type of organism as to dominate the bacteriological findings at this stage. The other groups represented only occasionally showed a significant number of organisms. A large number in the peptonizing group occurred only in butter showing very low scores.

The numbers of yeasts and oidia found, however, suggested a possibility of attaching some significance to this determination. The counts of these organisms in the entire series of butter samples were tabulated (table 1). The samples were arranged in the order of butter score, beginning with the highest scoring sample. The corresponding microscopic count of the cream used in making these samples of butter is introduced wherever it was obtain-

TABLE 1

Yeast and oidium counts in butter samples arranged according to commercial score

DESIGNATION	NUMBER	SCORE	MICROSCOPICAL COUNT		CULTURAL COUNT		MICROSCOPIC COUNT IN THE CREAM USED	
			Yeast	Oidium	Yeast	Oidium	Yeast	Oidia
Exp. past.....	93	92.7	215,000	<15,000	30	60	435,000	35,000
Exp., starter..	92	92.5	335,000	165,000	7,000	10,000	2,765,000	<15,000
Exp. past.....	90	92.0	135,000	<15,000	5	1	1,815,000	<15,000
Exp., cream, untreated...	88	91.5	35,000	<15,000	240	500	800,000	<15,000
Exp., cream, untreated...	91	91.5	435,000	100,000	1,100	7,000	1,685,000	<15,000
Cent., 1st.....	19	91.5	815,000	100,000	<10	<10		
Exp., starter..	89	91.0	300,000	<15,000	400	500	2,650,000	<15,000
Exp., neut....	100	90.5	400,000	65,000	50	40	585,000	165,000
Stored 1 yr....	31	90.5	300,000	<15,000	3	0		
Stored 1 yr...	30	90.0	465,000	<15,000	1	1		
Cent., 1st.....	13	90.0	985,000	15,000	1,100	<10		
Exp., neut....	99	89.5	50,000	30,000	40	20	1,065,000	265,000
Exp., neut....	39	89.5	55,000	<15,000	1	0	510,000	40,000
Renovated....	15	89.5	650,000	35,000	90,000	1,300		
Exp., neut....	63	89.5	335,000	35,000	4	6	285,000	15,000
Exp., neut....	98	89.0	580,000	230,000	20,000	12,000	3,035,000	450,000
Renovated....	74	88.5	250,000	<15,000	2,300	240		
Exp., neut....	38	89.0	95,000	<15,000	0	0	550,000	20,000
Exp., neut....	35	88.5	110,000	40,000	40,000	2,300	1,720,000	150,000
Cent., 1st.....	14	88.5	485,000	100,000	160	10		
Cent., 2d.....	20	88.5	1,315,000	35,000	<10	<10		
Exp., starter..	97	88.5	265,000	15,000	20,000	23,900	2,315,000	85,000
Renovated....	86	88.5	35,000	15,000	4,000	400		
Exp., neut....	81	88.5	165,000	<15,000	10	8	750,000	35,000
Exp., neut....	62	88.5	415,000	<15,000	7	3	900,000	<15,000
Exp., neut....	61	88.5	1,150,000	165,000	4,000	4,000	4,265,000	185,000
Exp., neut....	80	88.0	215,000	<15,000	260	40	865,000	<15,000
Exp., neut....	79	88.0	235,000	<15,000	24,000	9,000	3,515,000	385,000
Renovated....	75	88.0	85,000	<15,000	3,500	260		
Renovated....	73	88.0	65,000	<15,000	700	240		
Exp., starter..	36	88.0	425,000	40,000	2,900	400	1,815,000	95,000
Exp., starter..	60	88.0	665,000	200,000	13,000	7,000	2,535,000	315,000
Exp., past....	96	87.5	230,000	50,000	10	10	2,350,000	535,000
Exp. untr. cream.....	95	87.5	1,000,000	300,000	14,000	13,000	1,065,000	265,000

Abbreviations: Exp. = experiment; past. = pasteurization; Cent. = centralizer; neut. = neutralization; untr. = untreated; Starter-made from cream to which starter was added.

TABLE 1—*Continued*

DESIGNATION	NUMBER	SCORE	MICROSCOPICAL COUNT		CULTURAL COUNT		MICROSCOPIC COUNT IN THE CREAM USED	
			Yeast	Oidium	Yeast	Oidium	Yeast	Oidia
Exp. untr. cream.....	46	87.5	815,000	150,000	7,000	10,000		
Exp., starter..	78	87.0	400,000	165,000	8,000	11,000	2,435,000	550,000
Stored 1 yr...	57	87.0	150,000	50,000	8	1		
Stored 1 yr....	29	87.0	135,000	15,000	0	2		
Stored 1 yr...	28	87.0	85,000	15,000	10	30		
Exp., neut....	25	87.0	115,000	15,000	<10	<10	850,000	35,000
Exp., past.....	37	87.5	2,570,000	95,000	0	1	1,720,000	150,000
Exp., starter..	48	87.0	700,000	350,000	70,000	16,000	3,205,000	455,000
Exp. untr. cream.....	34	87.0	1,140,000	70,000	3,000	3,200	2,750,000	110,000
Stored 1 yr....	56	87.0	135,000	<15,000	11	0		
Exp. untr. cream.....	58	87.0	1,235,000	200,000	4,000	8,000		
Exp. neut.....	23	86.7	2,235,000	365,000	34,000	30,000	5,900,000	400,000
Exp., neut....	59	86.5	1,450,000	200,000	130	10	1,135,000	365,000
Exp., past....	77	86.5	365,000	50,000	70	22	4,135,000	750,000
Exp., neut....	51	86.5	150,000	35,000	1	24	700,000	220,000
Stored 1 yr...	41	86.0	20,000	<20,000	0	0		
Cent., 1st.....	17	86.5	1,400,000	100,000	1,200	800		
Renovated....	94	87.0	15,000	<15,000	900	40		
Stored 1 yr...	43	86.0	645,000	<20,000	0	0		
Exp., neut....	24	86.0	1,265,000	200,000	500	360	2,285,000	135,000
Stored 1 yr....	53	86.0	35,000	50,000	12	70		
Stored 1 yr...	42	86.0	620,000	70,000	0	0		
Stored 1 yr...	40	85.5	40,000	20,000	0	0		
Exp., neut....	50	86.0	385,000	135,000	3	4	1,140,000	205,000
Exp., past....	47	86.0	215,000	<15,000	50	20	2,475,000	425,000
Stored 1 yr...	52	85.5	65,000	15,000	16	80		
Stored 1 yr...	33	87.5	<15,000	<15,000	?	?		
Stored 1 yr...	32	85.5	15,000	<15,000	2	3		
Exp. untr. cream.....	22	85.0	750,000	115,000	11,000	1,400	3,285,000	450,000
Exp., neut....	49	85.5	350,000	50,000	12,000	18,000	1,830,000	260,000
Cent., 2d.....	18	85.0	850,000	50,000	160	80		
Stored 1 yr...	45	85.0	550,000	55,000	320	460		
Exp., 3d.....	27	84.5	1,235,000	335,000	<10	<10	2,415,000	400,000
Stored 1 yr...	55	84.5	685,000	385,000	6	0		
Stored 1 yr...	44	84.0	895,000	125,000	160	110		
Exp. untr. cream.....	76	84.5	1,900,000	235,000	25,000	14,000	3,300,000	715,000
Stored 1 yr...	54	84.0	1,715,000	285,000	12	0		

TABLE 1—*Concluded*

DESIGNATION	NUMBER	SCORE	MICROSCOPICAL COUNT		CULTURAL COUNT		MICROSCOPIC COUNT IN THE CREAM USED	
			Yeast	Oidium	Yeast	Oidium	Yeast	Oidia
Exp., starter..	26	83.5	800,000	150,000	3,000	21,000	3,235,000	150,000
Packing stock.	69	80.5	2,785,000	385,000	330,000	100,000		
Packing stock.	85	78.5	485,000	50,000	900,000	200,000		
Packing stock.	84	78.0	6,315,000	735,000	300,000	14,000		
Packing stock.	87	77.0	735,000	115,000	410,000	90,000		
Packing stock.	71	77.0	6,135,000	200,000	180,000	180		
Packing stock.	72	76.0	6 965,000	1,000,000	600,000	50,000		
Packing stock.	70	76.0	3,700,000	400,000	190,000	16,000		
Packing stock.	82	75.0	315,000	65,000	320,000	10,000		
Packing stock.	65	75.0	5,765,000	2,750,000	160,000	<10		
Packing stock.	64	75.0	1.785,000	1,185,000	38,000	37,000		
Packing stock.	83	74.5	565,000	400,000	22,000	270		
Packing stock.	16	74.0	1,215,000	1,250,000	6,800,000	330,000		
Grease butter.	68	74.0	1,885,000	115,000	3,600	200		
Packing stock.	67	74.0	1,065,000	300,000	200,000	24,000		
Packing stock.	66	74.0	2,735,000	715,000	120,000	25,000		
Packing stock.	21	73.5	4,750,000	4,015,000	500,000	700,000		

able. The butter investigated included but 3 samples scoring 92 or above. The other samples show progressively such lower scores as to justify the search for agents of deterioration.

From this table, the contrasts between the microscopic counts and the cultural counts in many cases represent the effectiveness of pasteurization of these samples. In general, low-grade samples show high counts of yeasts and oidia. Discrepancies occur frequently enough to indicate that other agents than these groups are capable of causing deterioration. No generalization is possible because this group of samples included too few high-grade products to establish a basis for comparison.

Yeasts and Oidium lactis spores are microscopically determinable in butter by the method proposed. The occurrence of these forms in both cream and butter which show signs of deterioration suggest the possibility of correlating the numbers of these organisms in the finished product with conditions in the cream used in manufacture.

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THE STAGE OF LACTATION AS A FACTOR IN THE VARIATION OF THE PER CENT OF FAT IN COW'S MILK

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It is in general known that there is a relation between the stage of lactation and the percentage of fat in milk. Pingree (1) concluded from a study of the advanced registry records of eighteen Guernsey cows that the percentage of butter fat showed, on the average, a tendency to increase throughout the entire lactation period but the increase was most marked during the first five months and during the last month. Van Slyke (2) found that the percentage of fat and protein dropped in the second month of lactation, as compared with the first, and then began to increase, continuing to increase from month to month during the entire period of lactation. In the tenth and eleventh months of lactation, the increase of fat and of proteins is more marked than during the preceding months. Eckles and Shaw (3) found in a study of eleven cows of four breeds that there is a decline in fat during the first three months, followed by a period of from four to five months with little change. From this period on to the end of the lactation period the fat increases rapidly reaching the maximum at the close. They also conclude that there is, in general, some relation between the amount of milk and the per cent of fat. When there is a sudden decline in the amount of milk secreted for any reason, as, for example, during sickness the per cent of fat with few exceptions increases sharply. The same holds true in the rapid decline that occurs in the last portion of the lactation period. Thorndike (4) found that the stage of lactation period makes a cow tend to vary from the general average composition of her milk as follows: First month 89.6, second month 90.3, third 92.4, fourth 96.5, fifth 97.9, sixth 101.2, seventh 103.2, eighth 103, ninth 104.3, tenth 104.9, eleventh 105.3, and twelfth 109.4. Grady (5) presents data on 10 Jersey

and 10 Holsteins showing the effect of the stage of lactation on both the yield of milk, fat, and the per cent of fat. He states that the fat content of the milk varied but little during the first four months. After the fourth month the percentage of fat gradually increased as the lactation advanced. The range in fat content of Jersey milk was somewhat greater than that of the Holstein.

The object of this paper is to present the relation between the stage of lactation and percentage of fat based on a study of 4045 records.

TABLE 1

*Influence of the stage of lactation on the percentage of fat in cows' milk**

MONTH OF LACTATION	GUERNSEY (3763) PER CENT FAT	JERSEY (299) PER CENT FAT	HOLSTEIN (95) PER CENT FAT
1	4.63	4.98	3.24
2	4.59	4.82	3.01
3	4.71	4.88	2.99
4	4.85	5.10	3.02
5	4.97	5.13	3.01
6	5.08	5.26	3.08
7	5.16	5.40	3.11
8	5.22	5.43	3.16
9	5.29	5.50	3.19
10	5.39	5.58	3.27
11	5.49	5.60	3.32
12	5.60	5.73	3.49

* Credit for the calculations involved in deriving the values in table 1 from the records is due the following students: Gerald Petty for the Guernseys, C. A. McCanse for the Jerseys, and J. E. Crosby, Jr., for the Holsteins.

The data presented in table 1, in the form of average percentages of fat, were derived from a study of the records of 3763 Guernsey Advanced Register tests, 299 Jersey Register of Merit tests, and 95 Holstein Advanced Register tests.

The Guernsey records were secured from the Advanced Register of Guernsey Cattle. The records published by the Guernsey Cattle Club are exceptionally valuable for a study of this nature as the itemized monthly productions of milk and fat as well as the average per cent of fat are published with each completed record. The animals used were representative with

respect to their ability as producers. The animals were scattered over the United States to some extent but the East and Central West where most of the Guernsey tests are being made was especially well represented.

Influence of the Advance of Lactation on the per cent of Fat in Cows' Milk.

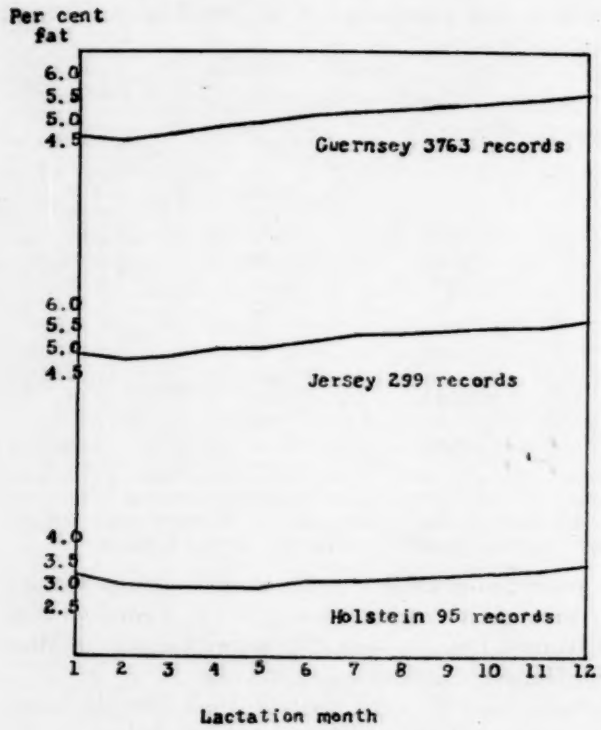

FIG. 1

The Jersey records were secured from the American Jersey Cattle Club. They consist of the records of Jersey cattle tested in Missouri during the past few years. All completed records available were used in this study so that animals of varying

producing ability and handled under varying conditions from the standpoint of management, feeding, etc., are represented.

The Holstein records were secured from the records of the Holstein herd owned by the University of Missouri. Here again the records used were of animals of varying productive ability and age but were made under similar herd conditions.

The data presented in table 1 and figure 1 shows that the per cent of fat during the first month is high and that there is a gradual decline in the per cent of fat, reaching its lowest point the second or third month. Following the low period there is a gradual increase in the per cent of fat. During the last month there is a more rapid increase in the per cent of fat up to the time the flow of milk ceases. It is undoubtedly true that there is some relation between the amount of milk produced and the per cent of fat (3–5). The sharp and more extended decline of the Holsteins as compared to the other breeds is probably due to the fact that the Holstein cows are generally tested for seven day records during the first month. This is usually as soon after calving as is permitted by the rules in order to secure the somewhat higher per cent of fat that may be expected from a cow in high condition at calving time. While it is customary to have cows that a e put on yearly test in good condition, the same attention is not given to having them excessively fat. Eckles (6) has shown that the per cent of fat in milk can be influenced to a marked extent for the first twenty to thirty days by the fatness of the animal at parturition. This influence appears to extend in some cases in a less degree for at least three months.

SUMMARY

It is shown that under conditions such as ordinarily prevail in the management of dairy cattle, and independent of the season of the year or character of the diet, the stage of lactation does have an influence on the per cent of fat in cows' milk. There is a noticeable decline in the per cent of fat from the first month to the second and in some cases this influence continues in a less degree for at least three months. Following the low period there

is a gradual increase in the per cent of fat which becomes more pronounced during the last month of lactation.

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COCONUT MEAL, GLUTEN FEED, PEANUT MEAL AND SOYBEAN MEAL AS PROTEIN SUPPLEMENTS FOR DAIRY COWS

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The dairy farmer of the corn belt can grow all the roughages necessary for his cattle and the best results are obtained when corn silage and clover or alfalfa hay are produced for this purpose. Grains of low protein content, such as corn and oats, are also easily produced in this section and the feeds that have to be purchased most generally are the concentrates of high protein content.

Much work has already been done in comparing feeds of this class so far as their value for milk production is concerned but in view of the newer knowledge of nutrition which demonstrates that the value of a feed may depend to a large degree on the nature of the other feeds with which it is fed it was felt that some additional work of this character need not necessarily be a duplicate of previous work. The basal rations used were constituted of materials commonly grown on corn belt farms, while the concentrates studied were ones on which little experimental data is available. The feeds studied were soybean meal, peanut meal, corn gluten feed and coconut meal, and they were compared with old process linseed oil meal.

Coconut meal. This feed, frequently known as copra meal, has been in use for a number of years on the Pacific Coast and during the war it seemed that the interruption of Germany's trade caused larger quantities of this feed to be shipped to the United States. There is now a fair trade in this commodity and it is handled by several distributing houses in the middle west. It is hardly probable however that large quantities will ever be available in this section.

Gluten feed. The increase in the manufacture of starch and glucose from corn has increased considerably in recent years,

and so larger amounts of the by-product gluten feed are becoming available. Much of this feed is produced in the corn belt, and though the greater part of it was for a long time shipped East, a considerable quantity of it is now used in this section.

Peanut meal. The growth of the peanut industry has been rapid and large amounts of the nuts are used for the manufacture of oil. Where the meal, that is left after the extraction of the oil, comes from hulled nuts, a feed of exceptionally high protein content is obtained. The meal from the unhulled nuts is known as peanut feed and is of considerably lower value.

Soybean meal. For a number of years soybean meal has been imported in considerable quantities to the Pacific Coast states from Japan, Manchuria and other regions in the far East. Freight rates prohibited the use of this feed in the middle west. Within recent years however the growing of soybeans in the South has increased rapidly and, as a large quantity of the beans is used for oil extraction, the soybean meal which is obtained as a by-product has been coming on the market in considerable quantities.

RESUMÉ OF PREVIOUS WORK

The compilation of a summary of work on a problem of this character tends to lead to confusion, as few direct comparisons of the feeds under consideration have been made and the conditions under which the trials have been made vary greatly. Consequently, it is necessary to consider the concentrates separately and also to give some attention to the work with cottonseed meal and oil meal in direct comparison as these two feeds have generally been the basal materials with which the newer concentrates have been compared.

Cottonseed meal vs. linseed meal

It was found by Waters and Hess (12) that cottonseed meal gives more milk and about the same amount of butterfat as oil meal, while Hills (5), Hunziker and Caldwell (7) and Michels (9) obtained better results with cottonseed meal than with linseed meal.

Cottonseed meal vs. other protein supplements

Both Scott (11) and Fitzpatrick (3) found coconut meal to be of about the same value as cottonseed meal, while Hunziker and Caldwell (7) stated that cottonseed meal was more economical than gluten feed. From their work Ewing and Ridgway (2) concluded that peanut meal was less valuable than cottonseed meal, while Gilchrist (4) found that soybean cake was slightly superior to cottonseed cake for milk production.

Linseed meal vs. other protein supplements

It was found by Hunziker and Caldwell (7) that gluten feed was more economical than linseed meal, while Hansen (6) found soybean cake and linseed cake to be of about equal value.

Gluten feed vs. coconut meal

It is stated by Lindsey (8) that coconut meal is of about the same value as gluten feed.

Gluten feed and peanut meal vs. no protein supplement

On replacing wheat bran and corn meal with an equal weight of gluten feed, Cooke (1) obtained an increase of 15 per cent in milk and 16 per cent in fat yield, while Pott (10) states that good results are obtained with peanut meal.

In briefly summarizing these results of the limited amount of work reported it may be said that on the whole cottonseed meal appears to give the best returns with linseed oil meal as a close second. There would also appear to be little difference in the values of the other feeds when compared with oil meal or with each other but that the addition of a supplement high in protein to a ration lacking such a supplement will increase milk and butterfat production.

EXPERIMENTAL WORK

The work reported here consisted of two trials of 150 days each in the first of which peanut meal and soybean meal were compared with old process linseed oil meal, while in the second trial coconut meal and gluten feed were compared with the linseed meal.

The analysis of the protein supplements used are given and for purposes of comparison they are arranged in order of their content of crude protein. All feeds were in good condition when used so any differences found cannot be attributed to variations in quality.

It will be noted that the peanut and soybean meals contain about 8 to 9 per cent more crude protein and the gluten feed and coconut meal 11 to 15 per cent less crude protein than does the oil meal.

TABLE 1

Composition of protein supplements

FEED	PEANUT MEAL	SOYBEAN MEAL	LINSEED OIL MEAL O. P.	GLUTEN FEED	COCONUT MEAL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Moisture	6.78	8.79	8.02	8.64	9.55
Dry matter	93.22	91.21	91.98	91.36	90.45
Crude protein	44.44	43.65	34.90	23.03	19.31
Nitrogen free extract	24.73	29.00	33.64	53.78	44.22
Crude fiber	10.63	6.12	10.82	8.07	11.71
Ether extract	8.76	6.28	7.32	4.21	8.73
Ash	4.66	6.16	5.30	2.27	6.48

Comparison of peanut meal and soybean meal with linseed meal

In this trial three cows were used. Previous to this work they had been handled with the general herd. During the experiment they were milked twice daily, allowed to exercise in yards whenever the weather permitted. They had salt before them at all times. They were watered morning and evening and were weighed before and after watering, so that a record of water consumption might be obtained. Hay, grain, and silage were each fed twice daily, the grain being fed on top of the silage. Some information concerning the animals used is given in table 2 and where necessary it is calculated to November 18, 1918, the day on which the experiment started.

The basal ration fed consisted of corn silage, alfalfa hay and a grain mixture of 2 parts cracked corn, 2 parts ground oats and 1 part wheat bran by weight. The roughages were fed according

TABLE 2

Animals used

	COW 253, GRADE GUERNSEY	COW 267, GUERNSEY	COW 284, JERSEY
Age, *years*	4½	4	3½
Fresh, *days*	72	140	109
Bred, *days*	0	0	0
Previous lactations	3	2	2

TABLE 3

Live weight, feed consumption, milk and butterfat production

PERIOD NUMBER	COW NUMBER	AVERAGE LIVE WEIGHT	TOTAL FEED CONSUMPTION				TOTAL PRODUCTION	
			Corn silage	Alfalfa hay	Grain mixture	Protein supplement	Milk	Fat
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
I. Oil meal	253	1019	600	160	120	60	353.6	16.99
	267	871	500	160	120	60	450.6	23.65
	284	888	500	160	140	70	481.2	28.24
	Group	926	1600	480	380	190	1285.0	68.88
II. Peanut meal	253	1035	600	160	120	60	332.7	16.22
	267	887	500	160	120	60	434.4	22.15
	284	890	500	160	140	70	465.1	28.60
	Group	937	1600	480	380	190	1232.2	66.97
III. Oil meal	253	1072	600	160	120	60	339.4	15.86
	267	906	499	160	120	60	426.1	21.20
	284	896	500	160	140	70	428.6	25.51
	Group	953	1599	480	380	190	1194.1	62.57
IV. Soybean meal	253	1070	500	160	100	50	326.3	14.93
	267	927	496	160	120	60	414.2	20.61
	284	891	500	160	120	60	405.1	24.81
	Group	963	1496	480	340	170	1145.6	60.35
V. Oil meal	253	1059	400	160	80	40	308.4	13.80
	267	937	500	160	120	60	417.4	20.04
	284	897	500	160	100	50	393.0	22.99
	Group	964	1400	480	300	150	1118.8	56.83

to the weight and capacity of the animals while the grain ration was controlled largely by production. Two parts of the grain mixture mentioned were fed along with one part, by weight, of the protein supplement being considered.

The experiment, of 150 days duration, was divided into 5 periods of 30 days each. The basal ration was fed throughout all periods while the protein supplements were old process linseed

TABLE 4

Peanut meal and soybean meal vs. old process linseed oil meal

PERIOD	AVERAGE LIVE WEIGHT	TOTAL PRODUCTION			TOTAL FEED CONSUMPTION			
		Milk	Fat	Fat	Corn silage	Alfalfa hay	Grain mixture	Protein supplement
Peanut meal vs. oil meal								
	pounds	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*pounds*	*pounds*
Oil meal.....	940	1239.6	65.72	5.30	1600	480	380	190
Peanut meal.....	937	1232.2	66.97	5.43	1600	480	380	190
Increase.....			1.25	0.13				
Decrease.....	3	7.4						
Increase percent..			1.9	2.5				
Decrease percent.	0.3	0.6						
Soybean meal vs. oil meal								
Oil meal.....	959	1156.5	59.70	5.16	1500	480	340	170
Soybean meal.....	963	1145.6	60.35	5.27	1496	480	340	170
Increase.....	4		0.65	0.11				
Decrease.....		10.9			4			
Increase percent..	0.4		1.1	2.1				
Decrease percent.		0.9			0.3			

oil meal in the first, third, and fifth periods, peanut meal in the second period and soybean meal in the fourth.

The first 10 days of each 30-day period was looked on as a preliminary period and the comparisons are consequently obtained from the remaining 20 days of each period.

In order that the protein supplements being studied may be compared with the oil meal, the results obtained with the supplement under consideration are compared with the average for the oil meal periods preceding and following it.

Throughout the periods where peanut meal and soybean meal were compared with the old process linseed oil meal the animals remained very uniform in live weight and the only variation in feed consumption was a difference of four pounds of silage between the soybean meal period and oil meal check periods—a difference which is negligible so far as its effects on the ultimate results are concerned.

The variations in production are also very small—indicating no practical difference in the value of the soybean meal and peanut meal for production purposes, when compared with oil meal.

Comparison of gluten feed and coconut meal with linseed meal

The outline for this work was exactly the same as that for the investigation with the peanut and soybean meal, with the exception that four cows were used. The data concerning these cows is outlined in table 5, and where necessary, calculated November 18, 1918, the day on which the work started.

TABLE 5

Animals used

	COW 289, JERSEY	COW 298, GRADE GUERNSEY	COW 301, GRADE GUERNSEY	COW 323, GRADE HOLSTEIN
Age, *years*	3½	3	3	2½
Fresh, *days*	200	166	239	180
Bred, *days*	12	40	15	57
Previous lactations	1	1	0	0

The basal ration used and the methods of feeding employed have already been outlined in the other part of the work and the results are summarized in similar form.

In this part of the work also great uniformity in the live weights of the animals was maintained. Slight variations in feed consumption did occur but all are negligible with the possible exception of decreases of 3.3 per cent in the basal grain ration and 6.5 per cent in the protein supplement consumed during the coconut meal period. This decrease was largely due to the relatively unpalatable nature of the coconut meal when compared

with oil meal which was exceptionally palatable to all of the animals used in the experiment.

TABLE 6

Live weights, feed consumption, milk and butterfat production

PERIOD NUMBER	COW NUMBER	AVERAGE LIVE WEIGHT	TOTAL FEED CONSUMPTION				TOTAL PRODUCTION	
			Corn silage	Alfalfa hay	Grain mixture	Protein supplement	Milk	Fat
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
I. Oil meal	289	773	400	120	120	60	342.8	21.50
	298	859	400	120	120	60	283.5	15.38
	301	779	400	120	120	60	369.1	19.83
	323	984	400	120	120	60	378.6	15.90
	Group	849	1600	480	480	240	1374.0	72.61
II. Gluten feed	289	778	400	120	120	60	323.9	21.87
	298	901	398	120	120	60	276.1	15.25
	301	799	400	120	120	60	348.9	19.88
	323	1000	400	120	120	60	345.6	15.03
	Group	870	1598	480	480	240	1294.5	72.03
III. Oil meal	289	792	400	120	120	60	291.9	19.99
	298	928	400	120	120	60	249.4	13.84
	301	814	400	120	120	60	342.5	18.50
	323	1018	370	114	111	51	290.6	13.87
	Group	888	1570	474	471	231	1174.4	66.20
IV. Coconut meal	289	811	398	113	103	43	255.8	18.75
	298	962	397	119	99	49	235.7	14.14
	301	836	400	120	120	60	317.5	18.41
	323	1020	393	117	80	40	270.1	13.46
	Group	907	1588	469	402	192	1079.1	64.76
V. Oil meal	289	819	400	120	120	60	281.8	19.23
	298	964	398	119	80	40	212.2	12.15
	301	827	400	120	120	60	347.1	17.88
	323	1014	400	120	40	20	222.3	9.46
	Group	906	1598	479	360	180	1063.4	58.72

When the production comes to be considered it is found that again there is little apparent difference in the value of the concentrates considered as protein supplements as the greatest difference in milk or butterfat production did not reach 4 per

TABLE 7

Gluten feed and coconut meal vs. old process linseed oil meal

PERIOD	AVERAGE LIVE WEIGHT	TOTAL PRODUCTION			TOTAL FEED CONSUMPTION			
		Milk	Fat	Fat	Corn silage	Alfalfa hay	Grain mixture	Protein supplement
Gluten feed vs. oil meal								
	pounds	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*pounds*	*pounds*
Oil meal	869	1274.2	69.42	5.45	1585.0	477.0	476.0	236.0
Gluten feed	870	1294.5	72.03	5.56	1598.0	480.0	480.0	240.0
Increase	1.0	20.3	2.61	0.11	13.0	3.0	4.0	4.0
Decrease								
Increase percent	0.1	1.6	3.8	2.0	0.8	0.6	0.8	1.6
Decrease percent								
Coconut meal vs. oil meal								
Oil meal	897	1118.9	62.46	5.58	1584	477	416	205
Coconut meal	907	1079.1	64.76	6.00	1588	469	402	192
Increase	10		2.30	.42	4			
Decrease		39.8				8	14	13
Increase percent	1.1		3.7	7.5	0.3			
Decrease percent		3.6				1.7	3.3	6.3

TABLE 8

Market value of feeds used

FEED	VALUE PER TON
Corn silage	$ 7.00
Alfalfa hay	25.00
Cracked corn	25.00
Ground oats	30.00
Wheat bran	40.00
Linseed oil meal O. P.	65.00

cent. It should be noted however that relatively the least milk and the highest yield of butterfat as well as the highest butterfat content in the milk was obtained when coconut meal was fed and it was not consumed in as large quantities as was the linseed meal.

Economy of production

Of greater importance than small changes in production is economy of production as it is on this point that the dairy business hinges. In arriving at some idea of the relative value of the protein concentrates considered it will therefore be necessary to give some consideration to cost of production. In deter-

TABLE 9

Value and feed cost of product and value of protein supplement

	TOTAL YIELD		VALUE OF PRODUCT	FEED COST LESS PROTEIN SUPPLEMENT				VALUE OF 1 TON OF SUPPLEMENT
	Milk	Fat		Corn silage	Alfalfa hay	Grain mixture	Total	
				Peanut meal vs. oil meal				
	pounds	*pounds*						
Oil meal	1239.6	65.72	$57.74	$5.60	$6.00	$5.70	$17.30	$65.00
Peanut meal	1232.2	66.97	58.53	5.60	6.00	5.70	17.30	66.00
				Soybean meal vs. oil meal				
Oil meal	1156.5	59.70	52.76	5.25	6.00	5.10	16.35	65.00
Soybean meal	1145.6	60.35	53.10	5.24	6.00	5.10	16.34	66.00
				Gluten feed vs. oil meal				
Oil meal	1274.2	69.42	60.64	5.55	5.96	7.14	18.65	65.00
Gluten feed	1294.5	72.03	62.64	5.59	6.00	7.20	18.79	67.00
				Coconut meal vs. oil meal				
Oil meal	1118.9	62.46	54.29	5.54	5.96	6.23	17.73	65.00
Coconut meal	1079.1	64.76	55.49	5.56	5.86	6.02	17.44	72.00

mining this the costs of all feeds except the protein supplements studied are taken at market value and the difference between the value of the products and the cost of all feeds except the protein supplement is taken as a measure of the relative value of the supplement for milk production.

The value of 70 per cent per pound is taken for the butterfat and $1.00 per 100 pounds for the skim milk and so with a value of $65 per ton for the old process linseed oil meal it is not difficult to get the relative values of the other protein supplements.

When the results are compared it is noticeable that all of the

protein supplements with the exception of coconut meal have a very similar value. Taking old process linseed oil meal at $65 per ton and using the method outlined, values of $66, $66, $67 and $72 per ton are obtained for peanut meal, soybean meal, gluten feed and coconut meal respectively. Apparently therefore soybean meal, peanut meal and gluten feed are of about the same value as linseed meal and coconut meal has a higher value than any of the others.

The results obtained are quite comparable with those found in previous work. There seems to be little doubt about the relative values of all of the feeds except coconut meal. It is not very palatable but seems to be quite valuable for milk production though the results obtained in its use have been at times contradictory.

Palatability of protein supplements

The palatability of all of the feeds under discussion has already been mentioned in another connection and during this work coconut meal was apparently the least palatable. The old process linseed oil meal was the most palatable, followed closely by soybean meal, while gluten feed and peanut meal ranked considerably lower.

None of the feeds appeared to have any specific effects on the animals as all kept in good condition throughout the work.

SUMMARY

The following deductions from the trial of protein supplements reported here are probably justified:

1. Old process linseed oil meal, peanut meal, soybean meal and gluten feed are probably of about equal value as protein supplements in the ration of the dairy cow.

2. Coconut meal may have a higher value, as a protein supplement, than the feeds mentioned, even though it has a lower content of total protein. The few pieces of work done on this problem are contradictory in character however.

3. In palatability the feeds studied rank as follows: linseed oil meal, soybean meal, gluten feed, peanut meal and coconut meal.

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FAT ANALYSIS OF MILK POWDER

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Up to the present time the Association of Official Agricultural Chemists has not designated an official method for determining fat in milk powders. The Roese-Gottlieb method, however, is generally accepted as the best method available for this purpose. Probably because of the fact that this Association has not officially adopted a method for determining fat in milk powder, and because of the urgent need for a method which is entirely reliable, numerous modifications of existing methods have been proposed. Some of these modifications have involved an extraction from an acid solution instead of an alkaline solution as is used in the regular Roese-Gottlieb procedure; others have involved modifications of the Babcock test, whereby the method could be adopted for factory use.

Biesterfeldt and Evenson (1) advocate the use of weak acetic acid, claiming this reagent allows extraction of fatty acids which may be present and which are not extracted from an alkaline medium. They claim the Roese-Gottlieb method gives an average of 0.04 per cent too low for condensed milk, and further state that for milk powders, the results may be even lower.

Harding and Parker (2) claim that more fat is extracted from evaporated milk by the use of 25 per cent acetic acid, carbon tetrachloride and petroleum ether than by the reagents used in the regular Roese-Gottlieb method. Biesterfeldt and Evenson (1), however, think that the higher results obtained by this method are due to the use of rubber stoppers in contact with the reagents.

Mohs (3) recommends a preliminary treatment of milk powder with hydrochloric acid and subsequent extraction in the Soxhlet apparatus with ethyl ether. He states that all of the fat is not

[1]Former Laboratory staff members participating in these comparative analyses were, A. H. Green and P. A. Bunce.

removed from milk powder by simple extraction with ethyl ether, and that there appears to be a decrease in fat from old powders due to absorption of the fat by the coagulated protein.

Phillipps (4) recommends extraction with trichlorethylene for rapid extraction of the fat.

Redmond (5) has recommended a modification of the Babcock test for factory use, the results from which are claimed to be from 0.1 per cent to 0.2 per cent higher than by any ether extraction method and 3 per cent higher than the regular Babcock method.

Roop (6) recommends the use of dilute sulphuric acid followed by digestion in the steam bath in the place of ammonia as used in the Roese-Gottlieb method for fat in dried buttermilk.

It seems to be recognized that a simple ether extraction in the Soxhlet apparatus is distinctly inferior to the Roese-Gottlieb method for liquid milk, evaporated, condensed or powdered milks. The majority of proposed modifications of methods have sought to improve the efficiency and reliability of the Roese-Gottlieb method. These modifications as well as the simple Soxhlet extraction method have but little recognition at the present time for determining fat in milk powder, although the Soxhlet method is quite frequently used for this purpose.

The inadequacies of a simple ether extraction for determination of butter fat in milk products were no doubt widely recognized after the official adoption by the Association of Official Agricultural Chemists at the 32nd Annual Conference, November 15, 1915, of the Roese-Gottlieb method for fat in milk and condensed milk, both sweetened and unsweetened. At the same time it was recommended by associate referee Hortvet (7), that a further study be made of this method in the analyses of ice cream, milk powders, malted milks and milk chocolates. This recommendation was, no doubt, stimulated by the relative accuracy and uniformity of results from evaporated and condensed milks. The results reported from a single sample of evaporated milk on which forty-five analyses were made by nineteen different analysts showed a maximum variation of 0.29 per cent. From two samples of sweetened condensed milk

analyzed by the same collaborators and involving twenty-five to twenty-seven determinations on each sample, the maximum variations were 0.21 per cent and 0.17 per cent.

At the Association meeting held November 20, 1917, Hortvet (8) reported results of collaborative work on analyses for fat in dried skimmed milk by the official Roese-Gottlieb method and by an acid extraction modification of the same. The results from the official method reported from a single sample on which twenty-five analyses were made by nine different analysts showed a maximum variation of 0.33 per cent on a powder containing 1.13 per cent fat as an average of all determinations. The results reported from the acid extraction modification showed a maximum variation of 0.65 per cent on the same sample. On the basis of these results it was recommended that further study be made of the Roese-Gottlieb method as applied to dried milk products containing a high as well as a low butter fat content.

COMPARISONS OF RESULTS FROM DIFFERENT METHODS

During our investigational and control work connected with desiccated milk, it has been desirable to make comparisons of different methods used for determining the butter fat content of this product. The results reported herein were obtained during a period extending over two years from dried milk made by the Just double roller process. The great majority of results, unless otherwise designated, were made with the Mojonnier apparatus which is considered a practical adaptation of the chemical principles of the Roese-Gottlieb method for routine factory analysis; those determinations made by this procedure will be hereinafter designated as the Roese-Gottlieb (Mojonnier) method.

VARIATIONS IN RESULTS OBTAINED BY THE ROESE-GOTTLIEB (MOJONNIER) METHOD

One of the first studies to be made in connection with this work on reliability of methods was for the purpose of ascertaining normal variations between duplicate determinations made by the Roese-Gottlieb (Mojonnier) method. The duplicate deter-

minations made by different analysts on samples of freshly made milk powder are shown in table 1. Since the two determinations on any one sample were made within short intervals, thereby eliminating the possibility of any discrepancy due to variable moisture content, the results are expressed on the original powder basis and not on the moisture-free basis. From the results shown in this table, the maximum variation between duplicate determinations by the Roese-Gottlieb (Mojonnier) method is 0.26 per cent; the minimum variation is zero; and the average variation is 0.099 per cent.

TABLE 1

Results of duplicate determinations for fat in milk powder, Roese-Gottlieb (Mojonnier) method.

SAMPLE NUMBER	DETERMINATION NUMBER 1, PER CENT FAT	DETERMINATION NUMBER 2, PER CENT FAT	DIFFERENCE, PER CENT FAT
1	23.93	24.19	0.26
2	24.47	24.51	0.04
3	26.55	26.55	0.00
4	31.12	31.12	0.00
5	28.12	28.02	0.10
6	25.68	25.47	0.21
7	27.21	27.39	0.17
8	27.07	26.95	0.12
9	28.33	28.29	0.04
10	30.09	30.05	0.04
11	26.21	26.32	0.11
12	19.75	19.84	0.09
13	53.51	53.65	0.14
14	55.00	54.93	0.07
Average.....			0.099

VARIATIONS IN RESULTS OBTAINED BY THE ROESE-GOTTLIEB (MOJONNIER) METHOD FROM FRESH AND OLD MILK POWDER

In order to determine whether or not the ageing of milk powder introduced a factor which would tend to cause discrepancies in the results from the Roese-Gottlieb (Mojonnier) method, several samples of powder made from part skimmed milk and from whole milk were analyzed while fresh and after various storage periods;

moisture determinations were made on each sample at the time of analysis and the fat percentages converted to the moisture-

TABLE 2

Results from Roese-Gottlieb (Mojonnier) method on part skimmed milk powder while fresh and at age of two months

SAMPLE NUMBER	FRESH	AFTER TWO MONTHS
	per cent fat	*per cent fat*
1	12.52	12.23
2	13.07	12.73
3	11.37	11.24
4	11.82	11.91
5	11.28	11.23
6	12.47	12.55
7	12.70	12.49
8	12.24	12.54
9	12.58	12.42
10	12.18	12.25
Average.....	12.22	12.15

TABLE 3

Results from Roese-Gottlieb (Mojonnier) method on part skimmed milk powder while fresh and at age of three months

SAMPLE NUMBER	FRESH	AFTER THREE MONTHS
	per cent fat	*per cent fat*
1A	12.70	12.69
2A	12.16	12.39
3A	12.58	12.60
4A	12.39	12.47
5A	12.27	12.18
6A	12.29	12.35
7A	12.39	12.14
8A	12.46	12.65
9A	12.23	12.34
10A	12.63	12.56
Average.....	12.41	12.46

free basis. The results of these determinations are shown in tables 2 to 5 inclusive.

In tables 2 to 5 inclusive there is opportunity to compare results from two determinations on thirty-five different samples, one

determination in each case being made while the powder was still fresh and the other when the powder was from two to six months old. The maximum variation between two determinations on the same sample is 0.41 per cent; the minimum is 0.01 per cent; and the average is 0.17 per cent. The results of these

TABLE 4

Results from Roese-Gottlieb (Mojonnier) method on part skimmed milk powder while fresh and at age of six months

SAMPLE NUMBER	FRESH	AFTER SIX MONTHS
	per cent fat	*per cent fat*
1B	12.82	13.08
2B	13.76	13.48
3B	12.33	12.18
4B	12.14	12.07
5B	13.23	12.84
Average	12.85	12.73

TABLE 5

Results from Roese-Gottlieb (Mojonnier) method on whole milk powder while fresh and at age of four months

SAMPLE NUMBER	FRESH	AFTER FOUR MONTHS
	per cent fat	*per cent fat*
1	26.72	26.56
2	27.34	27.32
3	27.76	27.41
4	27.09	27.16
5	27.44	27.32
6	27.41	27.23
7	26.31	26.51
8	27.93	28.25
9	27.48	27.81
10	27.79	27.38
Average	27.32	27.29

determinations show wider variations than was found in the samples recorded in table 1. This is undoubtedly due, to some extent at any rate, to the fact that the results from these four series containing thirty-five samples are all expressed on the moisture-free basis, the wider variations being explained in part,

as the result of analytical discrepancies in the moisture determinations.

In considering the question of the extraction of less fat from old powder than from fresh, there were nineteen samples from which less fat was extracted after ageing; the average difference was 0.183 per cent. The remaining sixteen samples gave higher results after ageing; the average difference was 0.154 per cent. Although the average difference between fresh and old samples shows that 0.029 per cent more fat was extracted from the former, this is not considered as conclusive evidence that this method of analysis is less reliable for old powders.

ROESE-GOTTLIEB (MOJONNIER) AND SIMPLE ETHER EXTRACTION METHODS COMPARED

Although the simple ether extraction method with the Soxhlet apparatus is not generally recognized as an efficient method for the determination of fat in milk powder, it is, however, frequently used in commercial laboratories for the purpose of determining the fat content of different consignments of this and other food products carrying a guaranteed fat content. In order to obtain comparative data between the two methods, parallel determinations were made on powder from part skimmed milk and from whole milk when the powder was fresh and after a storage period of four months. In the ether extraction method the extraction was carried out for seven hours. These results which are shown in tables 6 and 7 are all expressed on the moisture-free basis.

From the four different comparisons between the Roese-Gottlieb (Mojonnier) and ether extraction methods which are possible in tables 6 and 7, there was on an average 0.22 per cent more fat extracted by the former method. Each group of parallel determinations shows a similar relationship between the two methods.

Again considering the variations in results between individual determinations on the same sample as shown by both of these methods, it was found that from the Roese-Gottlieb (Mojonnier) method there was a maximum variation of 0.49 per cent; a minimum of 0.01 per cent; and an average of 0.143 per cent. From the simple ether extraction method there was a maximum

variation of 1.29 per cent, a minimum of 0.05 per cent and an average of 0.369 per cent. In these comparisons as in those

TABLE 6

Roese-Gottlieb (Mojonnier) and simple ether extraction methods compared on part skimmed powder, fresh and after four months

SAMPLE NUMBER	FRESH		AFTER FOUR MONTHS	
	Roese-Gottlieb	Soxhlet	Roese-Gottlieb	Soxhlet
	per cent fat	*per cent fat*	*per cent fat*	*per cent fat*
1	12.46	12.01	12.49	12.21
2	12.24	12.34	12.64	12.18
3	12.45	12.36	12.94	12.62
4	12.68	12.13	12.60	13.42
5	12.27	12.19	12.18	11.73
6	12.29	11.99	12.35	11.88
7	12.39	11.96	12.45	12.25
8	12.46	11.88	12.65	12.96
9	12.23	12.06	12.34	12.11
10	12.63	12.33	12.56	12.27
Average.....	12.41	12.12	12.52	12.36

TABLE 7

Roese-Gottlieb (Mojonnier) and simple ether extraction methods compared on whole milk powder, fresh and after four months

SAMPLE NUMBER	FRESH		AFTER FOUR MONTHS	
	Roese-Gottlieb	Soxhlet	Roese-Gottlieb	Soxhlet
	per cent fat	*per cent fat*	*per cent fat*	*per cent fat*
1	26.98	26.33	26.92	26.42
2	26.81	26.75	26.56	26.45
3	27.30	27.09	27.32	27.45
4	27.41	27.43	27.28	27.97
5	27.06	27.21	27.16	26.75
6	27.51	27.39	27.32	26.91
7	27.14	27.49	27.23	26.96
8	26.77	25.73	26.51	26.11
9	26.92	26.28	26.77	26.20
10	27.37	27.29	27.38	27.14
Average.....	27.12	26.89	27.04	26.83

already recorded there is no conclusive evidence that less fat is extracted from old powder than from fresh, regardless of the method.

EXTRACTION FROM AN ACID MEDIUM

Since it has been claimed that more fat is extracted from an acid solution than from an alkaline solution, comparisons were made between one of these methods and the Roese-Gottlieb (Mojonnier) method. The method used was the one recommended by Roop (6) for dried buttermilk in which powder is digested in dilute sulphuric acid (1:9) in the steam bath for thirty

TABLE 8

Roese-Gottlieb (Mojonnier) and alkaline and acid extraction methods compared

SAMPLE NUMBER	ACID EXTRACTION	ALKALINE EXTRACTION
	per cent fat	*per cent fat*
1	27.21	27.85
2	27.47	26.72
3	27.08	26.40
4	26.10	25.73
5	26.51	26.37
6	26.15	25.56
7	26.15	25.46
8	25.49	25.26
9	25.69	25.75
10	26.29	25.70
11	27.46	25.91
12	27.01	26.26
13	26.18	25.57
14	25.96	25.71
15	25.98	25.33
16	25.75	26.05
17	24.52	25.09
18	25.51	26.14
19	26.12	26.01
Average.....	26.24	25.94

minutes prior to extraction. This method as applied to the Mojonnier manipulation differs only in the fact that in the latter method ammonium hydroxide is used to digest the casein, whereas digestion is accomplished by weak acid and heat in the former method. The results obtained from these two methods are shown in table 8. The figures show that out of 19 comparisons, 14 are higher from the acid extraction method, and that the average difference is 0.30 per cent higher from this latter method.

ROESE-GOTTLIEB (MOJONNIER) AND REDMOND METHODS COMPARED

The results from the Redmond method, in which a given weight of powder is tested by the Babcock centrifugal method after a preliminary digestion with dilute sulphuric acid, have been compared with the Roese-Gottlieb (Mojonnier) method. From the results of these comparisons which are shown in table 9, it will be noted that the Redmond method does not compare in accuracy with the Roese-Gottlieb (Mojonnier) method. It would seem that this method can only be used as a rough control

TABLE 9

Roese-Gottlieb (Mojonnier) and Redmond methods compared.

SAMPLE NUMBER	REDMOND METHOD	ROESE-GOTTLIEB (MOJONNIER) METHOD
	per cent fat	*per cent fat*
1	33.30	28.77
2	28.08	28.55
3	28.08	27.40
4	28.08	27.24
5	27.36	26.85
6	56.88	55.41
7	55.62	54.02
8	56.39	54.08
9	55.98	52.81

method and even under these circumstances it appears to be of little value for close checking purposes. It is believed that the multiplication factor which must be used to obtain the final result is largely responsible for the discrepancies. The Redmond method results shown in table 9 are the averages of duplicate determinations. The maximum variation between duplicates of any one sample was 1.8 per cent.

SUMMARY

A review of the results recorded herein seem to warrant the following conclusions:

1. The normal variations in results from the same sample of milk powder analyzed by the Roese-Gottlieb (Mojonnier) method should not be over 0.15 per cent when results are expressed on the original powder basis. If results are expressed on the moisture-free basis, the variation between duplicate samples may be increased due to discrepancies in the moisture determinations.

2. There is no positive evidence of less fat being extracted from old milk powder than fresh powder when both samples are expressed on the moisture-free basis. If results are expressed on the original powder basis, there is an apparent decrease in fat content of old powders due to the fact that they may have absorbed moisture.

3. The simple ether extraction method gives results from the double roller process powder which are on an average about 0.25 per cent lower than the Roese-Gottlieb (Mojonnier) method. Duplicate results from the former method are also subject to wider variations than those from the latter method. From sixty-nine comparisons of duplicate determinations by the Roese-Gottlieb (Mojonnier) method there is an average variation of 0.147 per cent; a maximum of 0.49 per cent and a minimum of zero. From the ether extraction method from twenty comparisons there is an average variation of 0.369 per cent; a maximum of 1.29 per cent; and a minimum of .05 per cent.

4. On the basis of results presented herein, the Redmond modification of the Babcock centrifugal method is very unreliable. The wide variations between the two methods is undoubtedly accounted for to some extent by the large multiplication factor required for converting the test bottle reading to percentage fat in the powder.

5. The modified Roese-Gottlieb procedure from which extraction is made from an acid medium instead of an alkaline medium gave higher results in the majority of cases than did the regular extraction from an alkaline medium. The full significance of these higher results cannot be stated however, without further investigation as to the variability of numerous results from the same sample of powder.

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IS LIPASE A NORMAL CONSTITUENT OF COW'S MILK?[1]

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The evidence that a true lipase which accelerates the hydrolysis of milk fat is a normal constituent of cow's milk is not only inadequate but contradictory as well. It has been customary for most writers in reviewing this question to cite the experiments of Spolverini (1, 2), Marfan and Gillet (3), Moro (4), Gillet (5), Friedjung and Hecht (6) and Hippius (7) as having demonstrated the presence of lipase in milk, and possibly refer to the experiments of Vandevelde (8) who failed to find this enzyme. As a matter of fact, the examination of the original reports of these various investigators reveals the following facts:

Spolverini (1) states that he demonstrated lipase to be more or less active in the milk of the cow, goat, ass, and dog and also in human milk. Neither in this paper nor in the abstract of a later paper (2), where the same statement is made, is any experimental evidence whatever presented. The writer is of the opinion that Spolverini applied a test for esterase, previously worked out by Marfan and Gillet for human milk, to the milk of the animals stated.

Marfan and Gillet (3) added 10 cc. of 1 per cent monobutyrin solution and a few drops of alcoholic phenolphthalein to 1 cc. of cow's milk and after incubating for 20 minutes at 25°C. added sodium carbonate solution (approximately 0.5N) to the milk until it was neutral to the indicator. The lipase activity of the milk was reported as the number of drops of Na_2CO_3 solution required. This is stated to be 6 to 8 drops for cow's milk as compared to 20 to 30 for human milk under identical conditions. No antiseptics are mentioned as having been employed nor were

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any controls used. The conclusion is drawn by these authors that the presence of a monobutyrinase in cow's milk, which they believe they demonstrated, is not, however, an index of the presence of a true lipase. This statement of Marfan and Gillet seems to have been overlooked by most writers.

Moro (4) attempted to ascertain whether milk contains a true lipase which hydrolyzes neutral fat, but his experiments were with human milk only. The method was qualitative, and was rather novel, although its accuracy might be questioned. A small amount of neutral olive oil was added to the fresh milk in a small test tube and the mixture shaken gently, but not hard enough to cause a permanent emulsion. The tube was then incubated at 38°C. for a given period and the oil floating on the surface transferred to a 0.25 per cent sodium carbonate solution on a watch glass. If an emulsion occurred it was concluded that free fatty acids were present and the milk had contained a true lipase. According to Moro positive results were secured by applying this test to human milk.

Gillet (5) applied the method of Marfan and Gillet (3) again to cow's, goat's and ass's milk and also to human milk. His results, expressed in terms of drops of 0.5N Na_2CO_3 solution to neutralize the acid liberated from 10 cc. of 1 per cent monobutyrin solution by 1 cc. of milk after twenty minutes incubation at 25°C., were as follows: Human milk, 20 to 30 drops; cow's milk, 6 drops; ass's milk, 9 drops; goat's milk 2 drops.

Friedjung and Hecht (6) modified the method of Marfan and Gillet slightly, but applied it to human milk only. They concluded from their results that the evidence is conclusive that a monobutyrinase is present in human milk. The statement is made, however, that they did not attempt to ascertain whether a ferment is present which splits milk fat, also.

Hippius (7) attempted to demonstrate true lipase in human milk using a method similar to that employed by Moro, except that the free fatty acids liberated from the oil which was added to the milk were detected by means of an indicator. Positive results were secured by this method. No tests were made on cow's milk.

Vandevelde (8) carried out some careful experiments on the possibility of a true lipase being present in cow's milk. He rightly expressed the opinion that a study of the decomposition of fat in milk should be made on milk fat itself. He added 3.3 cc. of 3 per cent acetone solution of iodoform as antiseptic to 25 cc. of cow's milk in several tests from individual cows, and determined the increase in acidity after fifty-nine days in the incubator at 37.5°C. Acidities were determined (a) by steam distillation and titration of 50 cc. of the distillate after adding 50 cc. of alcohol, (b) by direct titration with 0.1N NaOH after adding 100 cc. of alcohol and 100 cc. of ether to dissolve both the fat and free fatty acids. In three experiments by the distillation method no increase whatever in acidity was noted, and identical results were secured in four experiments using the direct titration method. These results of Vandevelde's are open to the criticism that no study was made of the effect of the antiseptic employed on lipase activity. The writer has shown recently (9) that both acetone and iodoform have a marked retarding effect on lipase activity and if the solution of the antiseptic is slightly old so that a little free iodine is present, lipase activity is completely inhibited. More valid evidence of the absence of lipase in cow's milk was obtained by Vandevelde in another single test in which raw sterile milk, containing no antiseptic, was examined for free fatty acids in the extracted fat after having stood for fifteen months. The amount of free fatty acids in 5 grams of extracted fat was negligible, the fat requiring only 2.2 cc. 0.1N alc-KOH to neutralize the free fatty acids. Vandevelde drew the conclusion from his studies that the lipolytic decompositions which have been observed in cow's milk were the results of poorly conducted studies or failure to eliminate bacterial contamination.

From the experiments of Davidsohn (10) it seems doubtful, also, that cow's milk contains a lipase which splits even a simple glyceride like tri-butyrin. This investigator states that cow's milk can be differentiated from human milk by the fact that the latter will set free butyric acid from tri-butryin in a few minutes, while cow's milk or boiled human milk will not do so.

Another more recent experimental study of milk enzymes, which failed to demonstrate lipase in a product made from cow's milk, was that of Thatcher and Dahlberg (11). The curd of raw, fresh and stored butter was mixed with 5 per cent butter fat or olive oil, in the presence of 2.5 per cent chloroform, and the acidity while fresh and after four days' incubation, compared with that of control samples which had been boiled. No increases in acidity were noted which could be attributed to lipase activity. The experiment is open to the criticism that chloroform has a marked retarding action on lipase activity, as the writer has shown in recently published (9) studies.

Two studies which resulted in positive indications of lipase in cow's milk were carried out by Rogers (12) and later by Rogers, Berg and Davis (13). In the first study fresh cow's milk was mixed with an equal volume of heated butter fat and the initial acidity of the mixture compared with that after 13 and 19 days' incubation, using boiled fresh milk as control. An increase in acidity of 0.9 cc. of 0.1N acid per 100 cc. of milk was noted after nineteen days over the control. Formaldehyde 1:1200 was used as antiseptic. In another experiment Rogers divided fresh cream into two portions, heated one portion to 60°C. for fifteen minutes to destroy the lipase, if present, and churned both samples of cream. The butter was melted and salted in the melted condition and also treated with formaldehyde, so that the water in the butter contained 1 part in 1500 of the antiseptic. The acidity of the heated and unheated portions of butter after forty-eight and ninety-two days at 23°C. showed a small but distinct increase in the unheated portion which could be attributed to lipolytic activity.

In the experiments by Rogers, Berg and Davis, 50 cc. portions of milk which had been pasteurized at different temperatures were treated with 1 per cent $CHCl_3$ and 1 per cent ethyl butyrate. The flasks were neutralized at once to phenolphthalein using 0.1N NaOH and again at twenty-four hour intervals following incubation at 26°C. The results were compared with controls to which no ethyl butyrate was added. The results show that cow's milk has an appreciable ability to hydrolyze an ester like ethyl

butyrate under these conditions and that this ability is greatly weakened when the milk is heated to 66°C. (flash pasteurization) and destroyed at 80°C. In the writer's opinion these results should not be interpreted, however, as indicating the presence of a true lipase in cow's milk.

EXPERIMENTAL

In an examination of the possibility that cow's milk may, at times, undergo lipolytic fermentation which can not be attributed to bacteria or other contaminating organisms, the writer was impressed with the lack of definite data on the question whether cow's milk normally contains a true lipolytic ferment. The experiments regarding the abnormal condition referred to will be reported in another paper. In the present paper are reported the results of the study of a possible lipolytic ferment in normal milk.

Eleven experiments in all were carried out on this question. No foreign fats or single glycerides or esters were added to the samples under investigation because the problem involved solely the determination of a natural true lipase in the milk.

The technic used in the various experiments was not the same throughout the entire study. It was essentially identical for the first six experiments; it was varied materially in the seventh; and the last four experiments were performed using a still different technic. The experiments naturally fall, therefore, into three groups, altho experiment 7, alone, constitutes one group. It does not seem necessary to report each experiment in detail. The general features of each method will accordingly be described together with a summary of the results and the reasons for modifying the technic.

It may be mentioned that formaldehyde in the proportion of 1 part HCHO to 1500 to 2000 parts of milk, was used in all cases. The use of formaldehyde was based on experiments which have been reported elsewhere (9), showing that this antiseptic in this concentration has no detrimental effect whatever on the activity of animal lipase.

The source of the milk in all experiments was fresh milk from cows in the University herd. The milk in no case was more than a few hours old when the experiments were begun.

Experiments 1 to 6

In these experiments the technic employed was adapted from that used by Rogers, Berg and Davis (13) except that no ethyl butyrate was added. In detail, the method was essentially as follows in each experiment, each test being run in duplicate.

Method. Fifty cubic centimeter portions of milk and 25 cc. of sterile distilled water were introduced into sterile 200 cc. Erlenmeyer flasks. One cubic centimeter of neutral, saturated potassium oxalate solution was added to each flask to reduce the initial titration value and also to increase the sharpness of the end point, and the mixture then allowed to stand at least two minutes. Formaldehyde was next added to each flask in amount so that the concentration would be 1:1500 to 1:2000 after the initial titration had been made. Five drops of 1 per cent alcoholic phenophthalein solution were then added and each flask titrated at once to a two-minute pink with 0.1N alkali (aqueous). All flasks were incubated at 37°C. with frequent shaking, the flasks being stoppered with cotton plugs. The titrations were repeated at twenty-four-hour intervals for from two to five days in the various experiments. Each experiment included flasks containing (a) raw whole milk, (b) raw skim milk (prepared from the same milk by centrifugal separation), (c) raw whole milk heated to 80°C. for one minute, (d) raw skim milk heated to 80°C. for one minute. Either the heated skim milk or whole milk flasks were omitted in experiments 4, 5 and 6, one heated control being regarded as sufficient after it was found that there was no difference between the acidity which developed in the whole and skim milk samples after heating. In experiments 4 and 5 the whole milk was prepared by adding the cream from the centrifugal separation to portions of the skim milk.

The results of the experiments using this technic are summarized in table 1. The titrations have been calculated to cubic centimeters of 0.1N acid developed in 100 cc. of milk, which

could be attributed to lipolytic activity. In calculating the data the titration values obtained for the heated whole milks are subtracted from the titration values of the raw whole milks leaving the net acidities which could have developed in the whole milk as the result of lipase and other enzyme actions. Similarly, the difference between the acidities of the raw and heated skim milks gives the net acidities which developed in the milk plasma due to enzymes other than lipase. Where only one heated sample was run the result was used for both the whole and skim milk calculations. The assumption that the skim milks were free from fat was based on the fact that no demonstrable amount of

TABLE 1

Lipase activity of cow's milk as measured by direct titration at intervals following incubation

EXPERIMENT NUMBER	NET ACIDITY* IN WHOLE MILK AFTER			NET ACIDITY* IN SKIM MILK AFTER			ACIDITY* DUE TO LIPASE ACTIVITY AFTER		
	1 day	2 days	5 days	1 day	2 days	5 days	1 day	2 days	5 days
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	0.90	1.14	2.14	0.64	0.94	2.88	+0.26	+0.20	−0.74
2	1.96	1.60	2.35	2.06	2.76	3.64	−0.10	−1.16	−1.29
3	0.82	0.98	1.30	0.70	0.20	1.30	+0.12	+0.78	0.00
4	1.50	1.80	2.04†	1.86	3.00	3.24†	−0.54	−1.20	−0.96†
5	1.50	2.54	3.10†	1.34	1.74	2.30†	+0.16	+0.80	+0.80
6		1.00			0.80			+0.20	
7	2.72	2.10	2.60†	2.00	1.20	1.10	+0.72	+0.90	+1.60

* Cubic centimeters of 0.1N acid in 100 cc. milk.

† Three days in the case of experiments 4, 5, and 7.

fat was left in the skim milk after it had been passed through the centrifugal separator twice at high speed and a temperature of 95°F. The difference between the net acidities in the whole and skim milks gives the true acidity due to the action of lipase on the milk fat.

An inspection of table 1 shows very clearly that the presence of a true lipase in cow's milk is not demonstrated by the method employed in experiments 1 to 6. Whether these results are to be accepted without qualification, or not, will be brought out more clearly in the ensuing discussion and in the report of the other experiments.

The interesting feature of these experiments was the fact that acidities developed from day to day in the control samples, i.e., those which had been heated as well as in those which had not been heated. In order to throw some light on the cause of this phenomenon, some tests were made in imitation of the lipase experiments using, in one case, a solution of salts[2] which were intended to be a duplicate of the mineral salts of milk, both qualitatively and quantitatively, and using, in another case, serum from whole milk, prepared by filtering formaldehyde treated milk through a Pasteur-Chamberland filter under pressure. The following flasks were prepared and treated as in the case of the lipase studies:

Flask 1. 50 cc. salt solution + 25 cc. water + 0.5 cc. saturated potassium oxalate solution.
Flask 2. 50 cc. salt solution + 25 cc. water.
Flask 3. 50 cc. milk serum + 25 cc. water + 1 cc. saturated potassium oxalate solution.
Flask 4. 50 cc. milk serum + 25 cc. water.

The initial acidities and the acidities which developed on incubation are shown in table 2, the results being calculated in terms of cubic centimeters of 0.1N acid per 100 cc. The data in this table are not directly comparable with those given in table 1 where only the net acidities developed in the samples of milk are reported. It may be stated, however, that in general, the values given for the acidities developed in flasks 2 and 4, table 2, are about one-half of those obtained in the experiments using milk. A consideration of the data in table 2, together with this

[2] The salt mixture was that given for cow's milk by Van Slyke, L. L., and Bosworth, A. W., (N. Y. Agr. Exp. Sta. Tech. Bul. 39, 1914), and consisted of the following salts per liter of milk.

2.212 grams $CaHPO_4$, $2H_2O$
1.190 grams $CaCl_2$
1.030 grams $MgH_4(PO_4)_2$
3.040 grams $NaC_6H_7O_7$, H_2O
0.540 grams $KC_6H_7O_7$, H_2O

The $CaHPO_4$ was merely suspended in the solution, care being taken to mix it thoroughly before withdrawing portions for the tests.

fact, shows very clearly that the acidity which apparently develops from day to day when sterile milk is incubated is due in large part to a reversion of some of the salts formed in titration. Apparently this phenomenon can be prevented in part by precipitation of the calcium salts as calcium oxalate. This suggests that a part of the reversion to acid salts is due to the hydrolysis of $CaHPO_4$, which is known to occur, especially in the presence of alkali. This source of titratable acidity can be removed by oxalate. In all probability the $MgHPO_4$ which forms in the titration of $MgH_4(PO_4)_2$ hydrolyzes in the same manner. Magnesium salts, however, can not be removed by addition of oxalate.

TABLE 2

Acidity developed in milk salt solution and in milk serum on incubation

FLASK	CHARACTER OF MIXTURE	INITIAL TITRATION	ACIDITY DEVELOPED IN		
			1 day	3 days	5 days
		cc.	cc.	cc.	cc.
1	Salt solution, oxalated	8.90	1.40		2.94
2	Salt solution, untreated	11.80	1.80	3.80	5.50
3	Serum, oxalated	8.04	0.44	1.84	2.24
4	Serum, untreated	11.10	0.96	1.76	4.10

Experiment 7

The results secured in experiments 1 to 6, together with those obtained in the supplementary experiment on the cause of the acidities which developed from day to day in the control samples, prompted a careful consideration of the technic employed. This study led to the conclusion that the following serious criticisms could well be directed against this technic.

1. Acidity develops in both the experimental and control samples which is not due to enzyme action, and may vary with the amount of alkali added.

2. Neutralization of milk to a visible, satisfactory end point, using phenolphthalein as indicator, means that the relation of the milk is considerably on the alkaline side of neutrality, being at pH = 9 or over. This means that whatever lipase is present

in milk is being allowed to act in a hydrogen ion concentration considerably on the alkaline side of the normal hydrogen ion concentration of milk. Altho it is not known what effect this would have on the activity of lipase in milk, if present, the possibility of it being affected one way or the other is sufficient grounds for criticism.

3. If lipase is a normal constituent of milk, there is no basis for supposing that it exerts a selective action on the glycerides, liberating only those fatty acids which are soluble in an aqueous medium. Obviously the technic should be modified to permit the titration of both the water soluble and the water insoluble fatty acids which are liberated.

With these points in view another experiment was conducted in which the following technic was used.

Duplicate 250 cc. portions of milk were treated with formaldehyde 1:2000 and the initial acidity of each portion determined on a 50 cc. aliquot withdrawn with a pipette. The remainder of the milk was transferred to sterile 300 cc. Erlenmeyer flasks, stoppered with cotton plugs and the acidity determined on 50 cc. aliquots after incubation for twenty-four, forty-eight and seventy-two hours at 37°C. The acidity was determined in each case by adding 1 cc. of neutral, saturated potassium oxalate to the 50 cc. portion of milk, letting stand for several minutes, adding 250 cc. of neutral acetone-alcohol (1:1), filtering off the precipitate, after vigorous shaking, washing the filtrate several times with neutral acetone-alcohol (1:1), and titrating the combined filtrate and washings with 0.1N alcoholic KOH. In this experiment rosolic acid was used as indicator, but it was not found to have any advantages over phenolphthalein notwithstanding the fact that it changes color at pH = 7.

The results of this experiment, using the above technic, are shown in table 1, experiment 7, the data being calculated to values corresponding to the other experiments given in this table, taking into account the fact that the titrations of the aliquots after the twenty-four, forty-eight and seventy-two-hour incubations included the initial titratable acidity of the milk.

The data show that while the results in the last three columns indicate a slight development of acidity which could be attributed to lipase activity, at the same time it is apparent that this was due to an actual decline in the net acidity of the skim milk, and not to an actual increase in the net acidity in the whole milk samples. These data, therefore, like the data from experiments 1 to 6, do not indicate the presence of lipase in normal milk.

Experiments 8 to 11

In these experiments a somewhat different technic was followed in that in place of using boiled or heated milk as control samples, an antiseptic was added to the raw milk which had been found

TABLE 3

Lipase activity of normal milk indicated by comparison with milk to which lipase paralyzor had been added

EXPERIMENT NUMBER	ACIDITY* DEVELOPED IN MILK IN		ACIDITY* DEVELOPED IN CONTROL IN		ACIDITY* DUE TO LIPASE IN	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
8	3.74	2.30	−0.04	3.04	3.78	−0.74
9	−0.60		1.80		−1.20	
10(a)		0.60		−2.87		0.60
10(b)		2.44		−1.18		2.44
10(c)		3.90		−0.36		3.90
11		−0.56		2.96		

* Cubic centimeters of 0.1N acid in 100 cc. milk.

by experiment (9) to completely inhibit lipase activity. The antiseptic used for this purpose was iodine dissolved in KI. The skim milk controls were omitted, also, although the wisdom of this may be questioned inasmuch as results were secured in certain supplementary experiments in which a proteolytic enzyme was added to milk containing iodine, which indicated that proteolysis is not inhibited completely by this antiseptic.

Formaldehyde was used as bacterial antiseptic as in the other experiments and acidities were determined on fresh samples and after incubation as in the case of experiment 7. The technic

of this operation was modified to advantage in these experiments in the following manner. Saturated neutral potassium oxalate was added to the aliquot withdrawn for titration and after standing a few minutes was titrated to a pink color with 0.1N alcoholic KOH, using phenolphthalein as indicator. Two hundred cubic centimeters of alcohol-ether (1:1) were now added and the titration of the dissolved fatty acids completed. All tests were run in duplicate.

The milk used in experiment 8 was mixed milk from the University herd. In experiment 9 the mixed milk from two cows, only, a Jersey and a Holstein, was used. In experiment 10 the milk from each of three individual cows which had been in milk 411,429 and 390 days, respectively, was tested for lipase by this method. Experiment 11 was a repetition of the work on the milk of cow 502, tested in experiment 10, the retest being made thirty days later.

In calculating the results of these experiments in terms of possible lipase activity only the increase in acidity of the milk containing added iodine was subtracted from the increase in acidity in the milk to which no lipase paralyzor was added. The amount of iodine solution added varied a little in the different experiments, the idea being to furnish a surplus of free iodine after the iodine had reacted with all the casein in the milk. In experiment 8 the amount added was 8 cc. of 5 per cent solution in 5 per cent KI to 90 cc. of milk. In Experiments 9, 10, and 11 this was 6 cc. of 10 per cent iodine in 12 per cent KI.

The results of the experiments are summarized in table 3. Only in the case of Experiment 10 was any definite indication of lipase activity suggested. This was not confirmed in Experiment 11 using milk from the same cow a month later.

SUMMARY

Experiments are reported using several different methods of analysis which fail to show that cow's milk normally contains active lipolytic ferment.

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PROOF OF THE PRESENCE OF LIPASE IN MILK AND A NEW METHOD FOR THE DETECTION OF THE ENZYME

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There are now recognized several distinct enzymes which induce hydrolysis of fats and the simpler esters. These are grouped as lipolytic enzymes. The various members of the group are distinguished first according to their origin, for instance, the lipolytic enzyme of the stomach differs in properties from that of the pancreas (1) also, those from plants react differently from those of animal origin (2). Secondly, these enzymes are distinguished by the ester which each attacks most easily. Monobutyrinase is the name given to the enzyme which splits monobutyrin (3). The term esterase is used for that subgroup of lipolytic enzymes which split the simple esters more easily than the natural fats (4). The various members of this group may act on more than one ester but it is usually found that one ester is hydrolyzed more easily than another (5).

The term lipase has in late years been used to describe those enzymes which hydrolyze the natural glycerol, fats. They may also split the esters of the lower alcohols and acids, but they must act on the true fats to be known as lipases (4).

I. PREVIOUS METHODS FOR THE DETECTION AND DETERMINATION OF LIPASE

In most of the methods in use a convenient ester is chosen as substrate and after adding the extract to be tested, the mixture is titrated at the beginning and at the end of a period of time. The increase in acidity, which is due to the splitting off of acids by the enzyme, is taken as a measure of the lipolytic activity. When the digestion period is short no preservative is used, but if the time and temperature are such that the reaction is likely to be complicated by bacterial growth, then there is added a preservative to check such action.

1. The substrate

On account of the easy solubility of the lower esters, they have been most frequently used as substrates to test the action of lipase. Objection has been raised to this practice (6) since by definition lipolytic enzymes other than lipases may hydrolyze these. The difficulty in using the natural fats is in the fact that they are immiscible with water. The enzyme seems not to dissolve in the fat (7) but remains in the water layer, it can, therefore, act only on the fat at the interface. The action on the fat cannot be rapid unless it is emulsified in the water phase thus exposing a large surface of fat to the action of the enzyme (8). There has been much difficulty in preparing emulsions of the natural fats of such stability and uniformity that comparable results can be obtained at different times and on different enzyme extracts. In order to secure the desired emulsification of the fat there have been employed gums such as gum arabic, and also bile salts (9), but in some experiments dependence is placed merely on frequent shaking (10).

Aside from the simple esters of the lower alcohols and acids a great variety of which have been used as substrates (11), the following fats and oils have been employed rather commonly to determine lipolytic activity; cottonseed oil (12), castor oil (12), egg yolk (13), lard (10), cod liver oil (14), butter fat (15), almond oil (16), lecithin (17), olive oil (18), and linseed oil (19).

2. Methods of following activity of the enzyme on the substrate

As has already been mentioned the great majority of investigators make determinations by titration of the acid liberated from the esters and take these results as a measure of lipolytic activity. If aqueous alkali is used in this titration the results are likely to be too low when natural fats are employed on account of the fact that some of the fatty acids are insoluble in water and may not therefore react with the alkali. To lessen this error it has been recommended that alcohol be added to the solution to be titrated (20). This refinement has been frequently omitted by investigators, however.

Rachford (21) describes a method for determining lipolytic activity by using olive oil as substrate, and measuring the time necessary for sufficient fatty acid to be produced to permit easy emulsification of the mixture when brought in contact with a solution of sodium bicarbonate.

Where any salicylic acid ester is used as substrate, the salicylic acid liberated can be determined colorimetrically by use of ferric chloride (22).

If an ester is employed as substrate which splits into products that greatly alter the surface tension of the solution, then surface tension determinations can be made use of to follow lipolysis (23). Determinations of hydrogen-ion concentration (24), and of electrical conductivity (25) have also been suggested as means of following the splitting of fats by enzymes.

A unique method for detecting lipase was suggested by Carnot and Mauban (26), the fat is fixed in a solid media of agar and after the enzyme has been allowed to act at the surface, a color reaction with copper sulphate indicates that lipolysis has taken place.

3. The use of preservatives in lipolytic digestion experiments

Reactions brought about by enzymes and by bacteria are strikingly similar (27). Where enzyme digestions are proceeding in solutions suitable for bacterial growth the measurement of enzyme activity may be complicated by changes brought about by bacteria. Usually the substrate or the enzyme extract contains substances which bacteria break down; the temperature favorable for enzyme action is also very favorable for microörganisms. Commonly, preservatives are added which it is intended will not in the least check enzyme action but which will entirely prevent bacterial growth. A preservative which fulfills these requirements is not known, though there are a few that approximate the ideal.

In studies of enzyme lipolysis one of the first preservatives to be used was chloral hydrate (28) but it has been shown that this inhibits to some extent the action of lipase (29). Chloro-

form has been objected to on the same ground (30), though it has been used as an antiseptic frequently and has been particularly recommended for use in experimental work on milk enzymes (31); however, Vincent (32) found chloroform quite ineffective in preventing lactic fermentation. Kastle and Loevenhart (33) made an extensive study of the effect of common antiseptics on lipase, and found the greater number to be harmful.

It is significant that Vandevelde who is most frequently quoted in support of the hypothesis that milk contains no lipase (34) used iodoform in acetone as antiseptic. Yet it has been shown by Palmer (35) that lipolytic activity is nearly prevented by the presence of these substances. Toluol is probably the most commonly used antiseptic though formaldehyde has been found to have very little, if any, retarding influence on lipolytic hydrolysis (36).

In many of our preliminary experiments in which milk or butter fat was the substrate, bacterial action was not prevented by the liberal addition of toluol, chloroform, thymol or formaldehyde. In testing extracts high in lipase, and by use of such substrates as the simple esters, hydrolysis takes place so rapidly that there can be no confusion on account of bacterial action. However, such substances as milk which contain but small amounts of enzyme and where the natural fats are employed as substrates the hydrolysis proceeds so slowly and such a long digestion time is necessary (usually several days) that a thoroughly efficient antiseptic must be used. All media containing milk constituents are especially favorable for the activity of microörganisms.

Unfortunately much work has been done upon which reliance cannot be placed on account of the choice of preservative. Either it has been too weak to check bacterial growth or it has been of such nature that enzyme action is also inhibited.

Vandevelde (37) believed that cow's milk does not contain lipase and that the decomposition which others observed was due to improper control and especially to the presence of bacteria.

Saxl (38) concluded that none of the previously recommended methods affords a quantitative study of the ester splitting, since

the values obtained either are so slight on the short periods of digestion that they scarcely lie beyond the limits of error, or, on longer periods of digestion are obscured by the sources of error which are connected with the methods of determination.

Thatcher and Dahlberg (39) speaking particularly of lipase point out the "confusion which exists with reference to the normal enzymes of milk. The contradictory evidence which has been presented undoubtedly results in part, at least, from the rather crude methods of study of enzyme action which were in use at the time when those investigations were in progress."

II. PREVIOUS EXPERIMENTAL WORK ON THE PRESENCE OF LIPOLYTIC ENZYMES IN MILK AND MILK PRODUCTS

Marfan in 1901 (40) was probably the first to report that experimental proof had been obtained of the presence of a lipolytic enzyme which splits monobutyrin in the milk of woman and the cow. Detailed results were not published however until later (41). In these experiments monobutyrin was added to the milk and after a digestion period the acidity was titrated. It was assumed that any increase in acidity was due to butyric acid split off from the ester. The results can quite properly be objected to on the ground that no antiseptic was used to prevent bacterial growth; however the weight of evidence was greatly in favor of the conclusions reached.

Luzzati and Biolchini (42) and Spolverini (43) used the same substrate as Marfan and Gillet and followed a similar procedure except that after the digestion period the acid was distilled and the distillate titrated. They assumed that no volatile acid could be produced in the digestion mixture in any other way, even by bacteria, than from the action of lipolytic enzyme on the substrate setting free volatile butyric acid.

It will be noted that in the investigations just described the substrate used was always monobutyrin. Since it is understood in this work that lipase must split neutral natural fats, the presence of such an enzyme in milk was not necessarily proved. The methods which were used would indicate only one of the esterases—monobutyrinase.

Moro (44), on the other hand, employed olive oil as substrate and using the method of Rachford (21) found that milk was able to induce hydrolysis. This was the first time that a true fat-splitting lipase was identified in milk.

Friedjung and Hecht (45) demonstrated splitting of monobutyrin by milk. They used thymol as preservative and titrated both the digestion mixture and the distillate from same.

Gillet (46) found monobutyrinase in milk; he used as an antiseptic sodium fluoride and chloroform and titrated the acid split off directly without distillation. He obtained no enzymic splitting of triacetin or of a neutral fat substrate prepared from neat's foot oil, hence he concluded that no true lipase was present in milk.

Rogers (47) by preparing butter from fresh unheated cream and also by mixing raw milk with butter and adding formaldehyde as a preservative observed considerable increase in acidity which was not found in the heated check runs. He believed that this was due to the presence of natural lipase in the milk which hydrolyzed butter fat.

Hippius (48) using almond oil as substrate determined the lipolytic activity of woman's milk which had been heated to various temperatures. He made no attempt to estimate quantitatively the lipolytic activity but by use of an indicator particularly sensitive to fatty acids he determined whether or not the neutral oil had been split.

Koning (49) did not find the percentage of fat in milk to decrease on standing with an antiseptic, and concluded, therefore, that no lipase was present.

Vandevelde (37) using the fat of milk as substrate and iodoform in acetone as antiseptic allowed digestion to proceed for several weeks at incubator temperature. The acidity of the mixture and also of the distillate was determined at the beginning and at the end of the period. Since no increase in acidity was found he believed that no lipase was present.

Vincent (50) made a few determinations and was not able to prove the presence of fat-splitting enzyme in milk or cream.

Grimmer (51) found a monobutyrinase along with other enzymes in extracts made from the milk glands of the cow.

Davidsohn (24) used tributyrin as substrate and changes in surface tension as a measure of the splitting. There was added to the digestion media also disodium phosphate in order to preserve a reaction which he considered most suitable for the enzyme. He found very little lipolytic power in cow's milk but much more in woman's milk. He was unable to find a satisfactory preservative for use in the digestions and he admitted on this account that there was some weakness in his evidence, but believed notwithstanding this point that his conclusions were entirely warranted.

Thatcher and Dahlberg (39) used butter and olive oil as substrates and chloroform as antiseptic. They concluded that "lipases are present in butter in very small amounts, if at all."

Resch (52) followed methods similar to those used by Davidsohn but tested woman's milk only. The presence of lipolytic enzyme was found in all stages of lactation.

Palmer (53) has reported some investigations on the lipolytic activity of milk. His substrate consisted of an artificial milk prepared by emulsifying butter fat in water with gum arabic. Formaldehyde was used as preservative; acidity was determined before and after digestion by titration in presence of acetone and ether with alcoholic KOH. He states that "the work has not progressed to the point where it can be stated with assurance whether or not lipase is a normal constituent of milk."

Thus, it is seen that the question of the presence of lipase in milk has not been answered. While some investigators believe that they have established proof of an enzyme which will split neutral natural fat, others have been equally certain that milk has no such property, and many have been undecided even after careful experimentation. In most cases the methods used in testing for lipase are not free from objection, if no preservative has been added there is the possibility of a reaction taking place in the digestion media due to bacteria and not distinguishable from the enzymic reaction; on the other hand, if a preservative has been used there are the two possibilities that it may not have

been sufficiently powerful to check bacterial growth, or, it may have been so active as to inhibit the action of the enzyme also.

These remarks are to be understood to apply to true lipase. There has been an agreement with respect to monobutyrinase, no investigator having failed to find this enzyme.

In this connection should be mentioned the work of Pastrovich and Ulzer (54) who showed that fats were split under some conditions by proteins; casein was among those found to produce this effect. The theory that milk fat is split through the chemical action of protein was considered more or less favorably by Raudnitz (55). Falk (56) found a relation between lipolytic activity and chemical structure. However a careful inspection of the findings of all investigators on lipolytic action would indicate that in the large majority of cases, at least, the reaction is induced by a typical enzyme, rarely could the hydrolysis of the esters be attributed to chemical substances.

III. A NEW METHOD FOR THE DETECTION AND ESTIMATION OF LIPASE

From the discussion in the first part of this paper it is seen that the requirements of a dependable method for the detection of lipase are, (1) that a natural fat be employed as substrate, (2) that this fat be well emulsified in the aqueous solution, and (3) that a preservative be used which prevents the growth of organisms but does not inhibit lipolytic action.

Such an ideal method is very nearly reached in the following procedure: Cream of high fat (40 to 50 per cent) is employed. To this is added cane sugar in sufficient quantity to produce a saturated solution with the water present and with any water which may be later added in the enzyme extract. In order to bring this about, two parts of sugar should be added to one part of water.

The cream-sugar mixture is boiled to facilitate solution of the sugar and to destroy enzymes of the cream. Upon cooling, the milk or enzyme solution to be tested is added and the whole incubated at 38° to 40°C. for a considerable period of time. This may vary from three to thirty days or longer depending on the amount of enzyme present.

Acidity is determined at the beginning and end of the digestion period as follows: Ten grams of the sample is diluted with 50 cc. of distilled water. Neutral phenolphthalein is added and the mixture titrated to neutrality using 0.1N NaOH.

If the quantity of enzyme present is considerable, or, if the digestion period is sufficiently long, the characteristic odor of free butyric acid becomes evident.

The advantages of this method are that a neutral natural fat is used as substrate which is well emulsified in the water. The sugar increases the viscosity of the mixture to such an extent that there is no separation of fat, except after a very long time. However if such does take place, the mixture can be easily made homogeneous again by stirring. The sugar prevents bacterial growth but does not injure the enzyme nor prevent its action.

Although the effect of saturated sugar on other enzymes has not been investigated it would seem that the procedure applied here in the study of lipolytic enzymes might also find application in the study of many other enzymes where preservatives are necessary.

Experiment 1

The following is an example of the use of this method on the lipase of commercial pancreatin: One-tenth per cent of the powder was made up with water and then added to the cream-sugar substrate. The quantities of 0.1N NaOH necessary per 100 grams of the digestion media at various intervals are as follows: Beginning—4.0; three days—6.5; 7—8.5; 9—13.5; 13—22.0; 16—23.5; 23—26.5; 29—41.0; 77—86.5.

An accompanying check was run wherein the enzyme infusion was boiled. No increase in acidity was noted.

During the latter part of the digestion period a strong odor of butyric acid was evident. Bacterial count at the beginning of the digestion period showed 460 per gram, and at the end of twenty-nine days—90 per gram. In this determination room temperature was used for digestion and no effort was made to hold it uniform from day to day. This will account for the irregularity of acidity production at different intervals during the digestion period.

IV. THE DETECTION AND ESTIMATION OF LIPASE IN MILK

Experiment 2

To the regular cream-sugar substrate was added raw milk in different proportions. The temperature was again that of the room. The numbers of cubic centimeters of 0.1N NaOH required per 100 cc. of the digestion mixtures are here recorded.

TIME	PROPORTION OF RAW MILK ADDDD					
	1 per cent	3 per cent	5 per cent	10 per cent	15 per cent	20 per cent
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Beginning	3.5	3.5	4.0	4.0	4.5	4.5
8 days	4.5	5.5	10.0	12.0	13.0	16.0
16 days	6.5	10.0	11.0	14.5	15.5	20.0
22 days	6.5	10.0	12.0	14.5	15.5	24.5
29 days	6.5	10.0	14.5	18.5	17.5	28.5
36 days	6.5	10.0	13.5	16.5	20.0	22.0
43 days	6.5	9.0	12.0	15.5	19.0	25.0

During the latter part of the digestion period the odor of butyric acid was very strong in those samples containing the larger proportions of milk.

Raw milk is seen to be very active in the production of acid in the cream substrate even at room temperatures.

In the next experiment the samples were incubated at 38° to 40°C. under which conditions the enzyme is found to be much more active.

Experiment 3

Five per cent of raw milk was added to the regularly prepared cream-sugar substrate, and a check was run with an equal proportion of the same milk boiled.

TIME OF DIGESTION	0.1N NaOH PER 100 GRAMS OF MIXTURE	
	Raw milk	Boiled milk
	cc.	*cc.*
Beginning	5.0	5.0
8 days	14.5	5.0
14 days	20.1	5.5
20 days	22.0	5.5

The slight increase in acidity in the boiled check is due either to experimental error in reading the end point, or, to some change brought about by the action of the heat of the incubator on some of the constituents of the digestion medium.

At the end of the twenty-day period, the bacterial count on the raw milk mixture was 30 per gram. The bacteria present in this case and also in the case of the pancreatin digestion made in experiment 1 were doubtless inactive spore forms. For this reason and on account of further proof which is to follow it would not seem possible that they could have anything to do with the acid production, in which case it can only be concluded that there is an enzyme in the milk which is the cause of the change.

In order to show that the acidity produced in these digestions is due to a splitting of the milk fat the following experiment was run.

Experiment 4

A preparation was made by dissolving in skim milk sufficient cane sugar to make a saturated solution. This was divided and to each of two portions 5 per cent raw skim milk and 5 per cent of boiled skim milk were added. At the end of six weeks there was no increase in acidity. The same skim milk was found however to cause acid production when added to the cream-sugar substrate. These results show that it is only the milk fat which is acted upon by the active agency of the raw milk.

V. PROOF THAT THE INCREASE IN ACIDITY IS DUE TO ENZYMES AND NOT TO BACTERIA

The evidence thus far submitted points to the assumption that the active constituent of the raw milk is natural enzyme and that bacterial action has nothing to do with the acid production. The following points have already been offered in support of this conclusion.

1. The bacterial counts in all mixtures have been low. Especially should be noted experiment 1, where the bacterial count decreased as the acid production went on.

2. The rate of acid production increases regularly with the amount of raw milk added. If it were merely a case of inoculating the digestion media with bacteria then there would be much less difference where different amounts of milk were used.

3. The similarity of the action of raw milk to the lipase of commercial pancreatin is seen by comparing experiments 1 and 2. In addition to these experiments there have been run digestions by pancreatin and milk side by side. The action in these cases has been similar and particularly have they been found to behave alike when the acidity or alkalinity of the digestion media has been changed. There is every indication that milk lipase is identical with pancreatic lipase.

Rahn (57) in a very ingenious piece of work has shown how reactions brought about by enzymes differ in rate from those caused by bacteria. The reaction induced by enzymes is monomolecular, the greatest rate being found at the beginning and then gradually becoming less. On the other hand, when a reaction is brought about by bacteria there will be little action at first but as the numbers increase the rate increases. Of course later when the products of the reaction begin to accumulate the action again declines.

Thus, it is possible to ascertain if a given reaction is due to bacteria or to enzymes by determining the time at which the highest rate takes place. If it is at the beginning of the reaction it is certainly not due to bacteria. Bradley (58) has made measurements of the activity of pancreatic lipase, and has shown that a typical enzyme curve results.

Experiment 5

In this experiment 20 per cent of milk taken directly from the cow was added to the regular cream-sugar substrate; the temperature was carefully controlled at 40° and the acidity determined.

Following is the number of cubic centimeters of 0.1N NaOH required per 100 cc. of mixture at the beginning and each day for seventeen days thereafter:

Beginning—6.0; 1 day—9.0; 2 days—11.5; 3—14.0; 4—16.0; 5—17.0; 6—18.0; 7—19.0; 8—20.0; 9—22.0; 10—22.5; 11—24.5; 12—24.5; 13—25.0; 14—26.5; 15—27.5; 16—28.0; 17—30.0.

It is seen that the greatest activity is at the beginning of the digestion period with a slower rate thereafter. Plotting a curve with these results shows this very satisfactorily. This gives further proof that the reaction is not bacterial.

VI. LIPASE IN SEED OILS, BUTTER, CHEESE AND CONDENSED MILK

When disagreeable flavors develop in fats and oils and in products containing them, it is of considerable commercial importance since it may involve large losses. It is also of as great theoretical interest to learn the cause of such changes. Much work has been done on the subject yet there is but little agreement of opinion chiefly for two reasons: First, investigators are prone to draw conclusions from evidence which is at once insufficient and open to objection, and secondly, it has been difficult for one investigator to know just what another has meant when he describes these peculiar flavors. For instance, investigators formerly described as rancid all unusual flavors developing in fatty and oily products in storage. More recently particularly in dairy products "rancidity" has been used to describe only that odor resembling butyric acid, and the term "tallowiness" has come into use (59). Now no doubt many flavors which are really different are being described as tallowy. Therefore, it is not possible to judge as to just what condition is meant when an author describes his product as rancid or tallowy. In this discussion rancidity will refer to an actual fat hydrolysis which in dairy products results in the liberation of free butyric acid and the development of the odor characteristic of that substance.

It would not be safe to assume that all decompositions of fat substances in storage are due to the same cause, yet it is certain that if lipase is present in fat or oil that some change will take place. Breaking down of the fat in fat-bearing seeds and seed oils has been frequently attributed to lipase, often merely

by assumption but sometimes the presence of lipase has been directly proved (60).

The decomposition of fat in milk products is frequently noted. Whole milk powder develops a flavor on long storage which makes it impossible to be used as a food. Powdered skim milk does not change in this way which is proof that the change is connected with the fat. Since the flavor is never that of butyric acid but more like tallow, this condition should be called tallowiness rather than rancidity.

Both tallowiness and rancidity are found in butter. Without doubt true rancidity in this product is accompanied by the presence of butyric and related acids. It has been believed by some investigators that these acids are produced in butter by the action of bacteria on the lactose or casein. Some have concluded that certain species of bacteria either split the fat directly, or secrete lipolytic enzymes which do. Some of these assumptions have been shown to be possible (61). Air, light, and heat also bring about changes in fats and oils though the reactions taking place are much more complex than mere hydrolytic splitting (62), oxidation of the fats and later breaking down of the molecules probably are the chief factors.

On the other hand there are many things which point to the probability of the natural lipase of the milk being carried into butter and other milk products, there splitting off butyric and other acids from the fat thus leaving the product rancid. Rogers (47) offers some experimental proof of the theory. It has frequently been shown that butter made from pasteurized cream is of better quality than when prepared from raw cream. Also when butter or butter fat is strongly heated it is found that the keeping quality is enhanced and that it does not increase in acidity as does the unheated check sample. The fact that heating increases the stability of butter fat is in support of the theory of enzymes as a cause of deterioration.

Lipase of milk is quite easily destroyed by heat. Hippius (48) has shown that the enzyme is rendered inactive when heated at 64°C. (147°F.) for one hour. These results together with some

preliminary experiments in this laboratory indicate that the critical temperature for the destruction of lipase is very near to the pasteurizing temperature usually employed in factory practice. Since this is true and since in all probability natural milk lipase plays a part in the deterioration of butter, it is clear why some investigators have found pasteurized cream to yield butter of better keeping quality, and others have seen no benefit from pasteurizing. It is probable that in some methods of pasteurization the enzyme is not destroyed. That the temperature and method of pasteurization of the cream have a great deal to do with the quality of the butter is fully discussed by Hunziker (63).

The fact also that salted butter has been usually found to withstand storage better than unsalted (63) may also be used in support of the enzyme theory. According to Rogers the proportion of salt dissolved in the water of salted butter is about 18 per cent; it was shown by Terroine (64) that 3N sodium chloride (17 per cent to 18 per cent) reduced lipase activity about one-half. All lipase in butter would probably be in the water phase and would therefore be directly influenced by the salt.

Naturally any change which takes place in butter due to enzyme must be slow. All conditions are unfavorable for enzyme activity. The fat is not in emulsion form, and as has already been stated the enzyme probably acts only at the water-fat interface and since this is greatly reduced by the agglomeration of fat globules in the manufacture of butter, the attack of the lipase on the fat-substrate is correspondingly hindered. Butter is usually kept at a temperature below the optimum point for lipase, and as just pointed out the salt may hinder the activity. The amount of lipase in the milk and cream from which the butter is made may vary also. This would be reasonable to expect if the hypothesis of Grimmer (65) is correct—that the milk enzymes are excretory either being produced in excess in the body or coming from the food. With these points in mind it can be readily understood why investigators have reached such different conclusions concerning the effect of various factors on the keeping quality of butter. Some single factor unrecog-

nized and uncontrolled might be sufficient to throw the balance in favor of enzyme activity or against it.

It has also been pointed out that changes in cheese ripening may be in some measure due to natural milk lipase (66).

Rancidity in sweetened condensed milk is not infrequently encountered and the cause has not been definitely found (67). The following experiment would indicate that this condition is due to lipase of raw milk: To different portions of good sweetened condensed milk was added 1 per cent, 3 per cent and 5 per cent of raw milk. On standing three months all samples showed the typical odor and taste of rancid condensed milk, and were high in acidity, those with the higher percentages of added raw milk being the strongest. Checks with no addition and with boiled milk added remained normal to taste and odor. Bacteriological examination indicated that bacteria could not have been the cause of the rancidity. The high sugar content of the condensed milk is sufficient to prevent bacterial growth and the investigations recorded in this paper have shown that a high sugar content does not prevent lipolytic action. Further work is being carried on in this direction but the results so far indicate that sweetened condensed milk becomes rancid due to an error in manufacturing wherein a little raw milk may pass into the vacuum pan or the finished product, and that it is the lipase in this unheated milk that causes decomposition of the fat in the main body of the batch.

SUMMARY

1. All those enzymes which induce hydrolysis of fats and the simpler esters are grouped as lipolytic enzymes. Lipase is a term reserved for those lipolytic enzymes which split the natural neutral fats.

2. In the usual methods of determining lipolytic activity, a convenient ester or fat is chosen as substrate, the enzyme is allowed to act for a period of time and the extent to which the hydrolysis has taken place is measured by titrating the acidity. A few investigators have used other methods than titration for estimating the extent of hydrolysis.

3. If the substrate is not soluble in the digestion medium then it must be well emulsified.

4. If the time and temperature are such that bacteria can multiply, it is necessary to have present an antiseptic. The ideal antiseptic would be one which inhibits all growth of microörganisms but does not in the least check the action of the enzyme. The disagreement of investigators over the question of lipase in such substances as milk has been chiefly due to improper control of bacterial growth.

5. Investigators have been agreed as to the presence of an enzyme in milk which splits monobutyrin—monobutyrinase. Some believe they have established proof of the presence of true lipase, others have been certain that it is not there, while in recent years the tendency seems to be to leave it an open question.

6. A method for the detection of lipase is introduced, wherein is used cream of high fat content as substrate. The digestion medium is saturated with sucrose which serves as a preservative. Titration of the medium is taken as a measure of the hydrolysis. The advantages of the method are: a natural fat is employed which is well emulsified, the medium is preserved with a substance which prevents the growth of all bacteria that might interfere but which does not check enzyme action.

7. That milk normally contains a lipase which splits butter fat is proved. A few preliminary experiments indicate its resemblance to pancreatic lipase.

8. Where rancidity of fats and oils has resulted from hydrolytic splitting there is every indication that it has been caused by lipase. Since lipase has been proved to be normally present in milk, one of the causes of rancidity of butter and cheese is very probably the carrying over of this enzyme into the manufactured product.

9. Rancidity in sweetened condensed milk has been found to result when a small amount of raw milk is added to the product. This condition is doubtless due to the action of lipase of the raw milk splitting the fat of the condensed milk, the butyric acid resulting giving the characteristic disagreeable odor.

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STUDIES IN THE CONTROL OF A MUNICIPAL MILK SUPPLY

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The achievements of the past three decades in the fields of public health demonstrate the effectiveness of organized community efforts in the attainment of a heathful environment. One of the most important problems involved in municipal health administration is that of proper supervision of the supply of milk which is universally recognized as our most essential food.

The "four essentials" for the production of a clean milk of low bacterial content are: (1) Sterilized utensils; (2) small top milking pails; (3) clean milking animals and careful milkers; (4) adequate facilities for cooling and storage.

The veterinarian is an important factor for his advice in the care as well as in the testing of a herd; and the inspector is of tremendous value in emphasizing the need of cleanliness in milking, and the careful preparation of the milk by immediate straining, cooling and storage. It is obvious, then, that our information regarding the quality of a milk supply must be derived from a study of the bacterial content, coupled with inspection of the source. This is a problem of first concern, especially in our small cities. The milk supply of our larger cities, New York, for example, has, by careful supervision, improved so tremendously during the past few years that it is now usually found to be of a higher grade than that of our smaller cities. It has been demonstrated that milk of low bacterial count if properly cooled and placed in refrigerator cars may be delivered in good condition even after a haul across several states. Small cities, however, are not dependent upon milk from a distance but secure their product a few hours after milking, from close adjoining farms. Cooling and storage under such conditions will be neglected unless properly supervised.

Careful studies reported by the Virginia Agricultural Experiment Station (1911) (3), Brew (4) and North (2) have demonstrated that the standard score card is of practically no value in grading the quality of any individual milk because high grade milk may be produced from low-scoring dairies, the converse being equally true. The above observation has been further confirmed in a recent detailed study, over a period of thirteen months, of dairies supplying milk to a southern city of 37,500 population. It was found, for example, that a dairy averaging a score of 77 for the period of a year, produced milk showing bacterial counts which averaged 3,855,000 per cubic centimeter from analyses made almost weekly, ranging from individual results of 2000 to 72,000,000, whereas a dairy scoring 50½ gave one of the lowest results for the study, an average bacteria result for the same period of 250,000 per cubic centimeter, the minimum count being 1000, and the maximum 3,000,000. In the latter dairy personal interest and careful methods, factors essential to clean milk production, were highly developed, in spite of the absence of a new barn and modern equipment which the former dairy possessed. Scored on North's Score Card (2) which gives 90 per cent of the weight to the primary methods and equipment of milking, cooling and sterilizing, these dairy scores would have been adjusted to correspond more closely with bacterial results, the low-scoring dairy being raised from 50½ to 75.8.

In this study of municipal milk supply, eighteen dairies were under observation, of which number twelve were considered for the entire period from July 15, 1919, to July 31, 1920. Samples of the milk of the previous evening (stored according to a variety of conditions, sometimes iced, sometimes kept in spring water only, etc.) and of the morning's milk, which arrived within four or five hours after milking, were collected nearly every week. The samples were plated on agar according to the technique of "Standard Methods of the American Public Health Association" (1). The cleanliness of the milk was determined by the stain produced after filtering a half pint of milk through absorbent cotton discs, a perfectly clean milk scoring 100 per cent. Dairies were scored on the United States Department of Agriculture

Score Card by the city health officer, Dr. C. E. Smith, and the author.

Our studies of milk supply and dairying have led us to conclude with North that "There is no monopoly by model dairies of the sanitary methods vital to clean milk production, and consequently clean milk production is a possibility for even the poorest dairy farmer." One of the dairymen who produced the cleanest milk in this group reported kept a large herd of healthy cows, and with the assistance of his family was able to produce a clean milk with low bacterial content. He took personal interest in this milk production. On the other hand some dealers who owned dairies scoring among the highest found it necessary to employ several men, who of course did not take a personal interest in results; consequently it was impossible to produce a low count milk consistently. The "personal factor" in milk production is exceedingly important, for the care used by the milker in the dairy is one of the most important factors in the production of milk of low bacterial content. This is a factor which the present type of score card does not emphasize.

In order to obtain a low bacterial count, the dairyman must exercise care in certain essentials which have been previously suggested. The milk pails must be sterile, i.e., free from bacteria and dirt; the udders and flanks of the cow should be cleaned, preferably with a damp cloth, the air in the milking stable should be as free as possible from dust—feeding, sweeping, and passage of cows or other animals through the stable, or any other factor which would increase the amount of dust should not occur previous to or during milking; and the milk should be immediately cooled to 50°F. or lower, and kept cool.

While there may be no direct relation between individual dairy scores and bacterial counts, if we group the dairies equally according to scores we find that low-scoring dairies generally produce high count milk, as is indicated in the following table.

A similar grouping of the results of Joel (not published) (5) from a study of 125 dairies in Connecticut further confirms the above results as is shown by the following figures of average bacteria per cubic centimeter; 25,060; 27,600; 40,169; 58,950.

In other words, there is a fairly definite relation between equipment and methods and bacterial count. One seldom finds a dairyman who has dirty, unsanitary surroundings who is not careless in his methods. On the other hand, a dairyman who has a clean, modern barn and equipment is generally careful in his method.

It is not sufficient, however, for satisfactory milk supervision to employ dairy scores alone, but it is absolutely essential to carry on both inspection of dairies and laboratory examinations. Both of these are exceedingly important. Of the two, the latter is believed by the author to be of greater value for it is evident that the laboratory can test the milk of ten farms while the inspector is inspecting one.

TABLE 1

Bacterial counts and dairy scores of dairies under supervision, July 15, 1919, to July 31, 1920

NUMBER OF DAIRIES INSPECTED	DAIRY SCORES	AVERAGE BACTERIAL COUNTS	MAXIMUM DAIRY BACTERIAL COUNTS	MINIMUM DAIRY BACTERIAL COUNTS
3	77 and over	2,122,000	3,855,000	1,015,000
3	62–70	6,316,000	12,000,000	2,104,000
3	61–61.5	14,750,000	21,126,000	4,424,000
3	46.1–60	17,795,000	32,253,000	611,000

The bacteriological examination of milk is the most reliable index that we have of its quality, and with this aid we can generally show the dairyman the source of his trouble. Examples of this fact are common. One instance will be cited. In March, 1920, B. prodigiosus was found in the agar plates, and consistently thereafter through the month of June, from milk produced by a dairy excellently equipped and supervised. On June 24 the dairy was very carefully inspected. Previous suggestions for cleaning equipment had failed to eliminate this organism. At this time it was found that a milking machine had been installed on the first of March. Special recommendations for sterilization of the machine were made and the organism was not found again. Considering the fact that the milking machine was introduced previous to the appearance of B. prodigiosus, and that special

attention to other pieces of equipment failed to eliminate the organism, whereas particular care in the sterilization of the machine was followed by complete disappearance of the organism, it is thought that the milking machine may have been the source of the trouble. Special care in sterilization of the machine also lowered the bacterial content of the milk. In connection with the use of milking machines the recent work of Bright at the New York Agricultural Experiment Station (11) should be mentioned. He concludes that "while machines are quite complicated, yet clean milk can be secured with them if proper precautions are taken. These precautions are such that they come within the limits of the ability of every dairyman. The all-important principles which must be kept in mind is strict attention to detail."

If a laboratory examination consistently reveals high bacterial counts, or some other factor possible of correction, an inspection of the dairy and surroundings will generally suggest the source of trouble. "The laboratory test should come first and make the diagnosis; the dairy inspector should come second and apply the remedy." Problems connected with the production and handling or distributing of milk can be controlled by education better than through legislation. Rules and regulations are essential, but the gradual education by the inspector and health officer count tremendously. The progressive dairyman also comes to regard seriously his laboratory report, and to detect the cause of a high bacterial count or dirty disc.

In a study of the relationships of groups of bacterial counts to the cleanliness of the samples, the average bacterial count was found to vary inversely as the per cent of cleanliness; and in general in our results, samples showing a sediment test of from 80 to 89 per cent clean gave a bacterial count at least from three to four times higher than samples 90 per cent clean or better. Samples having a lower sediment test showed considerably higher bacterial counts. It should be stated in this connection that this *general* principle is of only limited application because it is noted that *individual* figures for cleanliness and bacterial count do not always show such a relationship, and a milk free from

sediment may give a high bacterial count and vice versa. Campbell, in 1916, in his study of various forms of sediment tests and their relation to bacterial count concluded that "The quantity of sediment or visible dirt present on the disc is not a criterion as to the kind or number of bacteria contained in the milk." This was a study of a small number of samples which were considered individually. Whereas the results obtained in the present study confirm this careful research when *individual* samples are studied, the additional fact of a general relationship of these two factors seems worthy of note.

One of the problems in the production of milk on the farm is that of proper cooling and storage. Pease (1916) (8) in a report on a study of the relation of high temperature of milk on receipt at the creamery to the bacterial content obtained concluded that the temperature of the milk as it was received at the creamery was not a safe indication of the bacterial count of the product contained therein. In studies supervised by Conn (9) on New York City milk it was found that milk properly iced showed no appreciable increase in bacterial counts over periods of even 48 hours. Reed and Reynolds (12), in a study of milk of various qualities with agar and gelatin media, found that many organisms are able to increase in numbers after long periods even at cold storage temperature.

Extensive studies have been made to show the effect of holding milk at different temperatures during varying lengths of time, the experiments having been carried on under ideal conditions. The results of Ayers, Cook, and Clemmer (10) indicate that even if milk when fresh shows a low bacterial count the number of bacteria will be high if it is held at a high temperature. This is of especial importance in relation to night's milk. The results of the authors just mentioned further indicate that the effect of temperature on the growth of bacteria in milk during storage and transportation is important. If a low count milk is desired it must be cooled and held at 50 degrees or lower on the farm, unless it is delivered immediately after each milking.

Results of a study of temperature in relation to bacterial counts are tabulated in table 2.

From the 780 samples of milk studied above, it will be noted that the percentage of samples showing bacterial counts over 500,000 increase and those under 100,000 and 25,000, respectively, decrease in a fairly regular manner as the recorded temperatures of the milk increase. These results were obtained from both morning and evening samples as well as a few mixed samples of milk studied. By classifying the morning and evening groups separately, similar results are obtained, even more strikingly for the evening's milk which was stored twelve hours longer than the morning's milk.

TABLE 2

Percentage classification of individual counts according to temperature of milk at delivery, in groups over 500,000, 100,000 and under, and 25,000 and under: July 15, 1919 to July 31, 1920

TEMPERATURE	NUMBER OF SAMPLES	PERCENTAGE OF COUNTS		
		Over 500,000	100,000 or under	25,000 or under
°F.				
40–44	67	13	77	59
45–49	76	19	73	56
50–54	184	21	58	31
55–59	162	22	58	31
60–64	162	32	47	26
65–69	46	32	54	23
70–74	39	33	48	28
75–79	13	30	61	7
80+	31	32	32	12

There is no relationship between temperature and average bacterial counts of raw milk from different dairies on account of the various elements besides temperature which have to do with obtaining a milk of low bacterial count, for even if exceedingly good precautions be taken for cooling, if careless methods have been employed or dirty utensils used, the milk will show a high bacterial count.

Those who have studied the milk question realize that the difference between morning and night samples may be very great, especially if both are collected at a milk plant at the same time. Milk inspectors, in collecting samples for examination,

are inclined to take only one sample from a dealer's milk, regardless of whether the sample taken be one of morning's or evening's milk. One cannot estimate the quality of a milk from the results of such a collection. This factor has been noted by Dearstyne and Jones (13), who suggest the necessity of taking a composite sample of milk when grading raw milk by numerical bacterial content. It was found in the present study of several hundred counts throughout the period of thirteen months that the bacterial results of morning and evening samples varied widely in most cases. Fifty-three per cent of all the dealers gave higher bacterial results for the *evening* average.

Evening's milk is usually cooled more carefully than morning's milk. This would tend to inhibit bacterial growth. The germicidal action in milk has been studied by Hunziker (1901) (14), Stocking (1904) (15), Heineman (1908) (16), Chambers (1920) (17), and others, who have found an actual decrease in raw milk under certain conditions during the first two hours after milking. (Chambers concludes that the action is specific, depending on both the individual cow and the species of bacteria.) The counts at the end of three hours are not generally much higher than at time of milking, but will develop rapidly under favorable temperature after this time; hence the necessity for prompt cooling and proper storage. As most of the morning's milk from the dealers here reported was four or five hours old at time of delivery and the temperature usually high, there was ample opportunity for some bacterial growth. Furthermore, the milk was only delivered in the morning and there was undoubtedly a tendency to haste in milking. Unless there be supervision, the morning's milk will not be produced under as careful conditions as the evening's milk. The factor of insufficient light may also play some part in a poorly equipped dairy, for sufficient light is necessary for careful technique in milk production, and dairymen generally begin work before dawn.

Table 3 indicates relationships which may exist between morning's and evening's samples of market milk, these results being averages for thirteen months.

This table is arranged according to the evening temperature, the milk showing most careful storage appearing first. In the first place, it is to be noted that the low counts in evening's milk and most of those in morning's milk appear in the group of dealers who cooled well the evening's milk. A study of the high counts which appear in these groups will be interesting. Dealer R.'s results show frequent high counts mingled with very low counts, indicating a laxity in methods or some like factor operating on these days. Most of the high counts from dealer O appear during the first few months when the dairy received little super-

TABLE 3

Bacterial counts of evening's and morning's milk with temperatures, July 15, 1919 to July 31, 1920

DEALER	MEAN TEMPERATURE OF DAY	BACTERIA COUNT OF EVENING MILK	TEMPERATURE	BACTERIA COUNT OF MORNING MILK	TEMPERATURE
F	67	335,000	47	137,000	65
R	66	8,549,000	47	910,000	57
J	63	438,000	50	638,000	59
D	65	592,000	51	1,923,000	55
O	65	2,793,000	51	3,992,000	63
H	77	32,584,000	54	12,447,000	67
C	68	28,502,000	55	35,998,000	68
A	65	4,237,000	56	13,163,000	61
K	63	1,325,000	56	191,000	64
M	65	24,571,000	56	6,561,000	68
G	74	1,282,000	58	3,906,000	58
B	63	2,825,000	59	950,000	61

vision and when the daily temperature ranged high. After August 8, 1919, temperatures and counts were very satisfactory. The samples from dealer H were collected during the warm months, as the mean daily temperature of 77° indicates. The dairy during this time was in a filthy condition, and the only good factor operating apparently was the cooling of evening's milk. Morning's milk was not cooled satisfactorily in more than 10 per cent of the cases. While dealer C cooled the evening's milk, he made no attempt to cool the morning product. Sterilization facilities were quite inadequate and careful methods were not consistent, there being very great fluctuation in counts. On

the other hand, the low-count milk came from dairies in which conditions were favorable because of especial interest exhibited by dairyman or of this factor coupled with excellent equipment. The factor of storage is of immense importance. In studying a table like the one above, however, one must consider the various other elements which have to do with obtaining a milk of low bacterial count, for even if exceedingly good precautions be taken for cooling, if careless methods have been employed or dirty utensils used, the milk must show a high bacterial count.

Our findings indicate that excellent results may be obtained from storage in tanks of water if these tanks are iced in summer weather. Insulated tanks are superior to others. Dearstyne and Jones found a much less increase in count of the product of a dealer who used an insulated vat and renewed his water supply than in case of the dealer storing his product in tubs of water, or in the uninsulated concrete vats in which no renewal of water was made. Kelly (18) states that a 10-gallon can of warm milk precooled with water at 55°F. and set in a tank of ice water at 37°F. can usually be cooled to 50°F. in about twenty minutes. The best and quickest way to cool milk is by use of a surface cooler with the coldest available water, then setting the cans in a well-insulated tank of ice water. Extreme care must be exercised in the cleaning of such a cooling apparatus. Gamble (19) found that felt jackets or insulated cans proved to be very effective in keeping milk cold during long shipments in hot weather and in preventing freezing during cold weather.

The seasonal variations in bacterial counts in milk have been frequently noted by North and others, who have observed that winter weather produces a reduction in bacterial counts. In table 4 there is presented the average monthly results of our study from July, 1919, through July, 1920. The mean temperature for the days on which milk was sampled were kindly furnished by the United States Weather Bureau.

A study of the above results indicates a rather striking seasonal variation in bacterial counts obtained from an average of *all results* from the dealers under supervision as well as from an average of the *maximum* and *minimum* counts for each month;

for all these counts become lower during the winter months, increasing again with the approach of hot weather. The column of mean daily temperatures is interesting in this connection, because in this way the possibility of warm days in the winter season (not uncommon in this locality) is taken into consideration. Another outstanding feature shown in the table is the appearance of uniformly high bacterial counts during the first two months of this investigation, those for July and August being enormous. For the corresponding month the year follow-

TABLE 4

Monthly results: Bacteria per cubic centimeter, average, maximum and minimum; temperature of the milk and mean temperatures of the sampling days, July 15, 1919 to July 31, 1920

MONTH	NUMBER OF SAMPLES	MEAN TEMPERATURE IN DAYS	AVERAGE TEMPERATURE OF ALL SAMPLES	AVERAGE BACTERIA PER CUBIC CENTIMETER OF		
				All counts	Maximum counts	Minimum counts
July	38	79	60	55,464,000	160,222,000	500,000
August	54	80	59	69,356,000	197,600,000	184,000
September	48	76	55	937,000	4,690,000	11,300
October	49	70	57	162,000	772,600	11,300
November	55	55	54	140,000	832,000	3,200
December	21	61	57	458,000	1,227,000	22,000
January	50	54	56	258,000	1,611,000	3,200
February	62	44	48	180,000	552,000	3,100
March	45	52	54	111,000	502,700	2,400
April	47	59	56	109,000	934,000	36,000
May	14	67	57	340,000	403,750	278,000
June	43	74	68	704,000	1,748,000	39,000
July	38	82	57	799,000	2,160,000	43,000

ing, the average counts do not even approach such high averages. Inasmuch as the results in 1920 are obtained from the same dealers studied in 1919, and practically the same number of samples were tested from each of the dealers throughout the year, it is safe to conclude that season is one important factor in the low counts of the cooler months. Furthermore, as there was no supervision of these dairies for a few months prior to July, and as the counts show such a decided drop and do not again return to such high proportions, it may be safely assumed

that the reduction of these high counts is due in a measure to supervision by laboratory analyses and dairy inspection. The ordinary dairyman, whether consciously or not, will strive harder to produce a milk of low bacterial content if he knows that his milk is subject to laboratory examination.

SUMMARY AND CONCLUSION

If the public health laboratory be used as an aid in determining the quality of a milk supply, and the results carefully analyzed in connection with the inspection of the source of supply, a reliable estimate may be obtained of the quality of the product from the dairy. The dairy inspections should be used entirely as educational measures. The laboratory indicates whether or not the milk has been adulterated and is an index of methods employed, while the inspection will place the blame where it belongs. In general, the better class of dairies produce the higher quality of milk, but this rule does not hold in all cases, for we frequently find a low-grade dairy producing a clean, low-count milk. In such cases the methods employed are good and offset the lack of equipment and sanitary surroundings. The tools of the laboratory and dairy inspection are invaluable for the health officer in the safe-guarding of a milk supply, and constant supervision of this nature will insure a more satisfactory product.

In the sampling of raw milk supplies, it is essential to collect samples both from the evening and morning product for a fair estimation of any day's milk. If careful supervision be given the milk production, and factors of cleanliness, sterilization, use of small-top pails, healthy cows and milkers, cooling and storage be employed, a consistently low-count milk may be produced. All of these factors are vitally important in the production of milk which must be stored for any period. Temperature has a very decided effect upon bacterial growth, and the bacterial counts invariably increase during the summer months in the milk of those dealers who do not carry out systematic cooling. Proper cooling, however, will maintain low bacterial counts. It has been found practicable by those dairymen who are personally interested in the production of a good milk supply to cool the

morning milk to 50°F. and maintain that temperature until delivery.

Many dairymen today have studied their problem, are well versed in the matters of health and sanitation, and desire to deliver a good product. This is especially true of those who maintain large routes in the cities. Many small dairymen, however, some of whom deliver their milk at wholesale to creameries and milk plants, are either new in the business or have never given serious consideration to the milk problem. If the health officer or milk inspector is tactful in approaching this type of dairyman, he may accomplish remarkable results in the way of education and response.

The best solution we have at present of the municipal milk problem is a grading system based on dairy scores and bacterial counts, together with pasteurization of all milk except that of the very highest grade.

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A COLOR TEST FOR "REMADE MILK AND CREAM"[1]

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Remade milk and cream are notable additions to our list of dairy products. The increased use of these products calls for a method of distinguishing them from natural milk and cream in order to protect the producer as well as the consumer against fraud.

A report (1) has already covered an investigation of several methods of attacking this problem. These methods were found to be of little or no value in detecting mixtures of natural and remade milk. A practical qualitative test for distinguishing natural pasteurized milk from mixtures of natural and remade milk is reported here. As little as 10 per cent of remade milk, varying somewhat with the grade of powder used, can be detected by this means. If condensed milk has been used in making the remade milk, the amount that can be detected will depend upon the degree of heat to which the condensed milk has been exposed in the process of manufacture.

According to Porcher (2) and Cazalas (3) no change has taken place in the casein of milk powder in the course of its manufacture except for a loss of sulphur. Jensen (4) states that the effect of heat on milk is to brown the casein, while Cazeneuve and Haddon (5), Leeds (6) and Hotz (7) claim that the lactose is altered. An increase in the titratable acidity of milk when exposed to a high degree of heat was noticed by Jensen (8) and Steinegger (9), while Van Dam (10) and Milroy (11) found an increase in hydrogen ion concentration. The action of caustic alkali on pure lactose was studied by Framm (12), Schade (13), Meisenheimer (14), Nef (15) and others, and its action on whole milk by Kruger (16), Gautier and Morel (17) and Grimmer (18).

[1] Published by permission of the Secretary of Agriculture. Presented at the 61st meeting of the American Chemical Soc., Rochester, N. Y., April 26–29, 1921.

In the course of experiments made in this Laboratory on the effect of alkali on the curd of milk, it was discovered that when the washed curd of the product made from milk powder was dissolved in sodium or potassium hydroxide, a yellow color developed in a few hours. It was also observed that the washed curd of natural pasteurized milk did not give this color.

It is known that pure lactose will give a yellow color when dissolved in caustic alkali. This is known as the "Moore test" (19). Five milligrams of lactose is sufficient to produce a trace of color in 10 cc. of 5 per cent sodium hydroxide. A yellow color is also produced by dextrose, fructose and maltose, but not by sucrose. The color is attributed to the aldehydic or ketonic nature of these sugars. It is also known (20) that aldehydes combine with proteins, probably with their free amino groups (21). According to Steinegger (22) the increase in acidity of milk when formaldehyde is added is due to a combination of the CHO group with an NH_2 group, with the elimination of water. It is conceivable that in the course of the manufacture of milk powder, lactose, being an aldehyde, might under the influence of heat and desiccation combine to a slight extent with casein and albumin in the same manner as other aldehydes. Experiments made in this Laboratory showed that there was a slight decrease in per cent lactose, as obtained by copper reduction, when milk was heated in boiling water for one and one-half to two hours. A slight increase in titratable acidity was also noticed which, however, may be explained in other ways (23). The yellow color produced by the action of alkali on the curd of remade milk, is, therefore, probably due to the presence in the curd of a compound of lactose of such a nature that it is not removed by washing. This compound has been formed presumably by the action of heat and desiccation in the process of manufacture of the milk powder and may possibly consist in a linking of the lactose with the protein, the aldehydic group of the lactose losing an atom of oxygen and the amino group of the protein losing two atoms of hydrogen.

The samples used in this investigation were made from skim

milk powder and unsalted butter as well as from whole milk powder and the components were so chosen as to bring the fat and non-fatty solids within the range for natural milk. Two types of emulsors and an homogenizer were used. Spray powders from eleven different manufacturers and drum powders from four different manufacturers as well as different samples and brands from the same manufacturer were used.

A few commercial samples of remade cream and ice cream in which milk powder had been used as well as 70 samples of market milk representing different distributors in the cities of Chicago, New York, Philadelphia, Baltimore, Washington and Richmond, were tested in the manner recorded here. Those samples which, from the evidence available, were known to contain remade milk or cream, responded to the test.

Experiments were made to determine the effect of heat on natural milk and it was found that in milk heated at the temperature of 70° to 72°C. for thirty minutes, the curd, when treated with alkali according to the method recorded here, gave a slightly different shade from that of milk heated at 63° to 65°C. for thirty minutes. The former temperature, however, is considerably above the holding temperature of pasteurization. A temperature of 80°C. for one minute was found to have no appreciable effect. It may be mentioned here that the peroxidase test was used in connection with these experiments and also on the commerical samples of market milk tested. This test begins to weaken or disappear at the temperature of about 72C°. All the samples of market milk examined gave a positive test for peroxidase. This test was made in two ways as follows: To 10 cc. of milk were added 5 drops of 1 per cent tricresol (24), 5 drops guaiac reagent (4 grams guaiac wood in 50 cc. acetone) and a drop of commercial H_2O_2. To another 10 cc. portion of milk were added 2 drops 1 per cent benzidine in 50 per cent alcohol, 2 drops 1 per cent α-napthol in 50 per cent alcohol and 1 drop of commercial H_2O_2. A blue color develops in the former case and red in the latter, provided the peroxidase has not been destroyed by heat.

METHOD FOR MILK[2]

To 25 cc. of milk in a 250-cc. beaker was added an equal volume of distilled water and after warming to 25° or 30°C. the curd was precipitated with 3.5 or 4 cc. of 10 per cent acetic acid. Distilled water to the amount of 200 cc. was then added and after standing for some time to settle, as much as possible of the supernatant liquid was decanted through a 166-mesh silk bolting cloth. The curd left on the cloth was washed back into the beaker. The beaker was again filled with water and the curd allowed to settle and decantation made as before. This was repeated three or four times. The curd was then transferred to a 15-cm. rapid double filter and washed at least three times, filling the funnel nearly full each time. The curd was broken up with a glass rod to facilitate washing. The filter was then removed from the funnel and after squeezing out the excess of water with the hand, the curd was placed in vials of clear glass 17 by 100 mm. and 10 cc. of 5 per cent sodium hydroxide added, the curd being broken up and mixed with the liquid with the aid of a glass rod. The yellow color began to develop in about two hours, the vials, however, were left over night and the final observation made the next day.

The control or standard for comparison was a sample of natural milk pasteurized at the temperature of 63° to 65°C. for thirty minutes. Several 25 cc. portions of this control were treated at the same time in exactly the same manner as the unknown and placed in vials of the same diameter.

METHOD FOR CREAM

To 15 cc. of cream was added an equal volume of water and after warming to 30° to 35°C., the curd was precipitated with about 2 cc. of 10 per cent acetic acid, filtered and washed. The greater part of the fat was then removed by washing first with 25 to 40 cc. of 95 per cent alcohol, then with 50 to 75 cc. of pure acetone using small quantities at a time, the curd particles being

[2] If the milk has been homogenized, the fat should be removed as in the method for cream.

broken up with a glass rod after each addition of the solvent. The curd was then washed thoroughly with water to remove the acetone and after draining was placed in glass vials of the same size as those used for milk, and 10 cc. of 5 per cent sodium hydroxide were added.

The standard used for comparison was a sample of natural pasteurized cream of about the same fat content. No attempt was made to detect mixtures of natural cream and remade cream.

SUMMARY

A practical qualitative method has been given for detecting remade milk and cream, based upon the color produced when the curd is dissolved in sodium hydroxide.

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METHODS OF CARING FOR MILKING MACHINE TUBES

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At a Milker Conference held at the New York Station on May 27, 1921, one of the most important points discussed was the relative value of the hot water method of sterilizing the teat cups and tubes, and the chemical method of sterilization by means of harmless chemicals such as brine and hypochlorites. Because the Station has frequently been asked to discuss its experiences in this matter, the following statement has been prepared.

The most natural thought in connection with the cleaning of any dairy utensil is that of scrubbing it, following this procedure with scalding water or flowing steam, and completing the cleaning process by drying. It is, therefore, not surprising that practically all of the early investigators of sanitary methods of cleaning milking machines (Harrison (6), Stocking (12), Stocking and Mason (13), Edwards (3), Meek (9), Haecker and Little (7), Harding, Wilson and Smith (5), Hoffman-Bang (8), Williams, Golding and Mackintosh (14), Burri and Höhl (2), and others) tried this method for milker tubes. Where the heat used was less than the amount necessary to kill microörganisms present, or where other sources of contamination were left uncontrolled, the results were very unsatisfactory. The bacterial counts under these conditions were usually high. Where sufficient heat was used to sterilize or to practically sterilize the various parts of the machine, the results reported are better and in several cases excellent. Thus for example, Edwards (3) reports counts as low as 1407 and 1776 per cubic centimeter when the tubes were thoroughly cleaned, boiled and steamed.

Practically all of the earlier investigators who have used heat-sterilization mention the fact that the rubber parts are so rapidly destroyed as to make this method of sterilizing milker

teat cups and tubes by boiling water or by steam impracticable under ordinary conditions.

With the development of the rubber industry and the development of heat resistant rubbers for use in surgical gloves, automobile tubes and other things, this situation has changed and there are today several rubber manufacturers ready to supply rubber parts for milkers that are designed to withstand boiling water or steam and that are at the same time sufficiently elastic to serve the purposes of mechanical milkers. This is particularly true of cloth-wrapped rubber tubing. Rubber parts such as are necessarily made in moulds are apparently not yet made of a truly heat resistant rubber; so that those milkers using moulded rubber inflations, or moulded rubber mouth pieces, are placed at a disadvantage in the use of the heat-sterilization method. Yet it may justly be held that moulded rubber parts for teat cups should not be discriminated against as it is usually so difficult to remove the straight rubber tubing inflations from the teat cups that companies using this type of inflations do not ordinarily advocate the complete removal of these inflations except for renewal, and provide for cleaning them without removal. Moreover, all persons familiar with the details of the matter seem to agree that even those machines which are normally equipped with heat resistant rubber inflations and tubes occasionally receive a lot of rubber parts from the manufacturers of these goods that are of very poor quality. This condition of affairs is apparently due to the difficulty that manufacturers of rubber goods have in supplying material of an absolutely standard and unvarying quality.

In view of the fact that several of the standard makes of milking machines in general use on dairy farms are not equipped with heat resistant rubber parts, it is unfortunate that sweeping statements have recently been published as to the general applicability of the heat-sterilization method for caring for the teat cups and tubes of milking machines, some investigators even asserting that this method of sterilization is the only method that can be used with good results.

Because of the difficulty in getting rubber goods of the right quality for use where heat-sterilization was to be used, investigators have from the first tried to secure satisfactory methods of sterilizing the rubber parts by means of chemicals of various kinds. Thus among the early workers in this field, Erf (4) tried boracic acid, a solution of lime, and formaldehyde. Stocking (12) and Stocking and Mason (13) tried brine, borax, lime water, formaldehyde, and soap powders, while Harding, Wilson and Smith (5) used brine. From this early work it became evident that brine was the most satisfactory solution that could be used for keeping the tubes sweet and clean, but its use was accompanied by the difficulty that some metals were corroded by it. Also, it was a preservative rather than a sterilizing agent. It was not until Ruehle, Breed and Smith (11) showed that the brine organisms were not capable of growth in milk and that milk organisms were not capable of growing in brine, and Wing (15) showed that brine could be readily and efficiently sterilized by the use of hypochlorites that the value of the brine-hypochlorite combination became really evident. Meanwhile an active advertising campaign was started by various firms selling hypochlorite solutions urging that milking machine tubes be disinfected by these solutions used alone. The net result of the latter campaign has been one of great disappointment. While milking machine tubes can be effectively sterilized by the use of hypochlorite solutions alone where these are used in sufficient strength and the strength is renewed with sufficient frequency, (Ruehle, Breed and Smith (11)), very few users of milking machines have appreciated the limitations of this type of sterilizing agent well enough to succeed continuously in producing a low count milk. In those cases where dairymen have neglected these solutions, it has not been uncommon, especially in hot weather, to find a man keeping his milker tubes in a stinking solution entirely free from any sterilizing agent and full of enormous numbers of organisms. Probably no one thing has so delayed the day when milking machine users will get satisfaction from their purchases and continuously produce a milk of good sanitary quality, as has this campaign of commer-

cial firms for the use of hypochlorite solutions. Hypochlorite solutions are highly effective for use with a preservative solution like brine; but when used alone, they require more attention than the average dairyman can or will give them.

However, the limitations of the brine-hypochlorite solution are such that no recommendation should be given for its universal use. The majority of the teat cups of milking machines are now made of metal parts that are not corroded by this combination; but in certain types of machines, because of mechanical limitations, this has not yet been accomplished with entire satisfaction.

A third method of caring for milker tubes has also been used quite commonly and with fair success in New York State where really cold springs are quite common. This method consists of allowing cold water to circulate through and over the milker tubes and cups between milkings. It depends for its success upon general cleanliness and the retardation of bacterial growth through the effect of cold and is successful only when low temperatures (preferably less than 50°F.) are maintained. Because this method of caring for the teat cups and tubes can be used without injury to rubber parts or to the metals ordinarily used, it has been tried in some cases where conditions did not justify its use. It is not a positive method of sterilizing in that no bacteria are killed, and results can never be made as perfect as in those cases where proper heat methods or chemical methods are used. Nevertheless, there are some men who are getting good results with it in New York State under farm conditions.

With all of the procedures that have been suggested for caring for the tubes there has been a common tendency for dairymen to rely either on heat or cold or the action of the chemical solution to destroy the bacteria, to the neglect of actual cleanliness. Milk has been allowed to dry onto the interior of the tubes, the teat cups claws have been allowed to become badly clogged with milky accumulations, check valves on the pail cover have been left uncleaned, tubes have been thrown carelessly into the solutions so that entrapped air prevented the action of the sterilizing solution, and many other details have

been neglected, with the thought that the heat, cold or chemicals took care of everything.

It was shown early in milking machine investigations that the tubes and cups could be kept in clean and sanitary condition without taking them apart daily if washed thoroughly by drawing an abundance of cold and hot water containing alkali cleaning powders through these parts *immediately* after each milking (Harding, Wilson and Smith (5)). The publication of this statement has been used as an excuse by milker salesmen and dairymen for saying that all the cleaning necessary was to draw a pail of cold water through the tubes whenever convenient after milking. In many cases this is all the cleaning milking machines have received for months at a time. This has been the case in spite of the fact that even where the tubes are cleaned thoroughly after each milking, there is always some blackening of metal parts where these are in contact with the rubber so that to keep them really clean and shining, they must be taken apart and each part individually polished at least as often as once a week. The failure of milker companies to teach the purchasers of their machines good cleaning methods and the failure of dairymen to realize the necessity for making satisfactory provision for proper conveniences for cleaning their machines had led to the production of large quantities of milk containing excessive numbers of bacteria.[4] Largely for this reason, with the return of more abundant farm labor, the hand milker has again become a severe competitor of mechanical milkers. It is therefore not surprising to find the various milker companies coöperating in pushing a vigorous campaign for the better care of their machines by dairymen.

Recently some public health authorities, because of a very natural and well grounded prejudice against the use of chemical sterilization of dairy utensils, have threatened to forbid the use of chemical sterilization for milking machines. Fortunately, so far as known to the writer, this policy has never been put into force, and it is to be hoped that it will not be. Health authorities have every reason to be active in compelling dairymen to produce a clean milk containing few bacteria or discard their

machines; but any attempt to enforce such a regulation as indicated would take us back to the days when dairymen were instructed by control officials that clean and sanitary milk could only be secured in whitewashed barns, with a specified number of windows and so on. Under present conditions, if health authorities or investigators dictate what method of cleaning milkers shall be used, or state that only one method is successful, because of mechanical limitations in the construction of milking machines, it gives the support of public agencies to one group of milker manufacturers as opposed to a second group. If there were any danger involved which affected the public health such a course might well be justified, even though one group of commercial interests were favored; but there is no evidence at present available that indicates the presence of such a necessity.

Some investigators have not realized the difference between the use of the ordinary chlorine solutions and the use of the brine-hypochlorite solution, or have reported that the latter method was not successful because they knew of instances where dairymen had reported that they were using it, yet the results secured were unsatisfactory. In the latter cases (as in the instances reported by Bright (1)) an investigation would undoubtedly show that while the dairyman may honestly think he is following the directions, he is failing to observe some essential step in the procedure.

Inasmuch as it has been amply shown that the chief source of the bacterial contamination of milk is from the dairy utensils with which it comes in immediate contact, (Prucha, Weeter and Chambers (10)) and as literally tens of thousands of dairymen in the United States, are using milking machines, the matter of a campaign for better care of these machines is highly important to all of the parties concerned. The public is interested through its agents, the public health authorities and experiment stations and colleges. The dairymen themselves are interested because any tendency to lower the quality of dairy products injures their business. The milker companies likewise face the necessity of showing that milking machines are capable of giving satisfac-

tion in the hands of all, or practically all, purchasers of their machines, or their business will disappear. Now that proved and tried methods of cleaning milkers are known, it would appear that the time is ripe for more vigorous campaign measures by all of these forces to improve a situation that is not what it should be. The milker companies, as already indicated, are individually or collectively organizing campaigns along lines that should command the support of everyone. Some states, such as New York, are carrying information directly to dairymen through extension activities. Some city milk inspectors and inspectors of dairy companies are securing correct information regarding these things and carrying it to the dairymen with whom they come in contact, and some of the dairymen's organizations are taking a real interest in encouraging their members to produce better quality products. Proper coördination of these activities would hasten the day when the users of milkers will clean and sterilize them properly. In New York State gratifying results are already evident from coördinated efforts along these lines so that we already have milk plants where educational measures have so reduced the trouble from dirty milking machines that many users of machines are continuously securing premiums at Grade A milk stations for the production of milk with a bacterial count less than 10,000 per cubic centimeter.

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OUTLINE FOR A STUDY OF THE COST OF MILK PRODUCTION

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More studies of the cost of production in the dairy business have probably been made, than in any other phase of agriculture. Yet the dairy farm is one of the most complex of agricultural businesses. The reason for the great number of investigations into the dairy business is mostly that, many farmers felt[1] they were losing money on their dairies, and the dairymen were among the most vociferous of all farmers in demanding higher prices, or some sort of a change in the conditions of the business so that their dairies might become paying propositions.

The writer has examined critically, the methods and the conclusions of over twenty investigations into the cost of milk production. These reports or bulletins cover practically all the important milk producing sections in the country. It was found that the methods of collecting the data, the methods of computation, and the systems of conducting the studies were almost as many as the reports. On a few of the more important and outstanding factors in the production of milk, the different bulletins were pretty well agreed, but as regarded the greater number of factors, very few of these bulletins could be compared directly, one with another. A careful study of a whole bulletin, and of the methods of collection and tabulation of data was always necessary, in order to understand fully the significance of its conclusions on any particular factor of cost.

The writer has endeavored to pick out, from among these studies, the methods of collection of data and of computation of each factor which seemed most reasonable, and which seemed to be based on the best business practice for the farmer.

In making a study of the cost of milk production, the herd will generally serve as the basis for study and comparison. The cost

[1] Particularly from 1916 to 1920.

will generally differ more between cows in the same herd, than between dairies, but one of the principal aims of such an investigation is generally to compare and to choose from among the different systems of management, and this can be done only when the dairy rather than the cow serves as the basis for comparison. Hence, it is the comparison of dairies rather than of cows that is considered in the following pages.

COLLECTION OF DATA

There are several possible methods of collecting data for an investigation of the cost of milk production, but the principal ones may be grouped under two headings; the Survey Method, and the Cost Accounting Method.

The survey method

The survey method is the one which will generally be used when the nature of the study does not allow enough time, or when the financial provisions do not permit of a sufficient expenditure for the use of more careful and accurate methods of collection.

Under the survey method, a comprehensive questionnaire is first drawn up, and a corps of men who are thoroughly acquainted with the dairy business, are employed to visit the farms. These men, by conversing with and questioning the farmers get the information necessary to fill out the blanks.

A great deal of care must be put on the questionnaire before any data is collected. Some of the principal points to be borne in mind, are the following:

1. The questions must be so clear as to be proof against misunderstanding or confusion on the part of the dairymen.
2. They must be so formulated that there will be no possibility of their antagonizing the farmer.
3. The questions must be in the farmers' vernacular, and free from scientific abstractions.
4. The questionnaire should cover every factor of the business that could possibly influence the facts which are being sought.

5. At the same time, useless questions must strictly be kept out, and the questionnaire should not be so long as to tire the farmer before he has discussed all the important factors.

6. Where it is possible, check questions should be incorporated in the questionnaire, in order to verify important points.

The man who is collecting the data, must always be able to supplement the farmers' answers with observation. He must be able to use tact and diplomacy in questioning further, on points where the farmers' answers appear to depart from the facts.

In the survey method, the data from individual dairies may have a considerable factor of error, because of poor accounts, and the general proximation of answers. However, if a sufficiently large number of dairies be visited, the errors from some may be considered as offset by errors in the opposite direction from others. This is only a rough and approximate sort of a tendency, but it may be depended on to secure reasonable accuracy in a study of a large number of dairies.

The cost accounting method

This is essentially the method used in cow testing associations. It has the advantage of greater accuracy than the survey method but the disadvantage of greater expense, and usually of a decided limitation on the number of dairies from which data can be collected.

Under this method, it is necessary to keep accurate accounts for each dairy, to weigh milk and feed, watch closely the time spent on the dairy by the farmer and his hired men, and in short, to check up all factors of expense and income. It has seldom been found possible to depend on the farmers to do this work for themselves with any degree of accuracy. The best method, and the one generally used to reduce expense to the bureau making the study, and to insure accuracy and uniformity of data, is to organize so-called Cost of Production or Cow Testing Associations, in which the farmers agree to pay the salary of an experienced man who will spend at least one day a month at each farm. There it is his duty to check up the farmer's accounts, weigh milk and feed, test milk, etc. It is not necessary to go

into the details of organizations here. They are widely known, and though they vary in details, their general scheme is pretty uniform, and their principal aim is to collect more accurate data regarding the operation of dairies than the dairymen could or would be likely to do for themselves.

Among the greatest advantages of these associations are that they permit an accurate inventory to be taken at the beginning and end of the year, and facilitate the keeping of a fairly complete set of accounts. For accuracy these accounts can best be kept by the central bureau or experiment station, the farmers mailing in a complete report of the farms' or dairies' operations each day. This is entered under the proper accounts by the central bureau. The books are then balanced at the end of the year, and the complete and fairly accurate data on each factor then becomes available.

As was mentioned above, the principal disadvantage of this system, aside from the expense, is in the limitation of the number, and also of the type of dairies from which data can be obtained. In the first place; one route man cannot attend to more than twenty-five dairies. And for a comprehensive study, data from two or three hundred should be available, or in other words, from eight or more associations.

Only the most efficient and profitable dairies of a given neighborhood are likely to enter such associations, indeed, only they will be likely to pay the necessary expenses of the cow testing associations. This will leave a large proportion of the dairies out of the study, and the most unfortunate thing is that, these dairies will comprise more or less definite classes which should be compared to the more efficient ones in the association.

The cost association method is by far the more accurate, but its conclusions, based on the association records, must always be definitely limited to the types of dairies actually found in the associations, and not stated as general or sweeping conclusions.

CLASSIFICATION AND COMPARISON OF CLASSES

After the data are collected, it becomes necessary to divide them into groups or classes in two directions; first, in such a way

that all the data in any one class will be homogenous, that is it must be from dairies of similar types, operating under similar conditions, in a restricted area, and producing like products. Secondly, the data must be classified in such a way as to facilitate the study of the particular items under discussion.

HOMOGENEITY OF DATA

The business of dairying is widely affected by the size of dairy, by the type of farming common in a given neighborhood, by the proximity of markets, the disposition of the products, the physical features of the locality, and by a number of other factors. Hence, if such a study is to be of any real significance, it must be confined to a sufficiently small area so that the dairies will all be comparable. It must further, contain a clear classification of dairies according to the accompanying system of farm management, size of dairy, disposition of product, efficiency of management, etc. The extent to which this classification should be carried will depend on the aim of the study, and the factors to which particular attention is being paid. To compare New England dairies directly with dairies located in Wisconsin, to include in the same table large and small dairies, or dairies selling "A" grade milk with those making butter, is to invite error. And to draw any but the most general conclusions from such data is fallacious and may be even harmful.

FACILITATION OF THE STUDY

The final comparison in a cost of production study will usually be based on the averages of the different classes of dairies from which have been drawn the data at hand. Considerable care must be taken in forming these classes in order that the averages may be comparable.

There should be a sufficient number of dairies in each class so that errors in collection and tabulation may have a chance to counterbalance, and in order that exceptional cases will not unduly influence the averages of the classes of the conclusions of the study. A class which contains fewer than ten or twelve

dairies will not fulfill this condition, and no very sweeping statement should be based on such a classification.

The ideal situation would, of course, be to so classify the dairies that only two factors would vary from class to class—one being the variable and the other the factor in which it is hoped to trace the effect of changes of magnitude in the variable. But it would be both difficult and expensive to collect data from a sufficient number of dairies so that each class or group contains dairies, which differ in two factors only, and agree in each of the twenty or more other factors of production. Therefore, it may sometimes be necessary to include in a class, dairies which agree in the most important factors but differ in a few minor ones besides that particular factor which is being treated as a variable. In this case the dairies should be so arranged that variations resulting from differences in minor factors will counterbalance, and so will not affect the results.

The comparisons between the different classes will usually show themselves as correlations. The correlations may be discovered by inspection, by the value of a factor rising or falling consistently from class to class as the independent variable is increased or diminished. The simplest correlation would be between two factors, the one being treated as the independent variable, which is the basis of classification and hence increases at a given rate from class to class. The other, or dependent variable, will show correlation if it increases or decreases at some ratio corresponding to that of the former. For instance, in studying the relationship between the production per cow and cost per quart; production per cow may be used as the independent, and the cost per quart as the dependent variable. The dairies might be classified as follows:

Class I, Dairies producing 2000 to 2999 pounds per cow
Class II, Dairies producing 3000 to 3999 pounds per cow
Class III, Dairies producing 4000 to 4999 pounds per cow
Class IV, Dairies producing 5000 to 5999 pounds per cow
Class V, Dairies producing 6000 to 6999 pounds per cow

Now the dairies are grouped under these classes with regard to production only, but if a correlation exists the average cost

will be found to rise from class to class as production is increased. Or if an inverse correlation exists, the cost will fall from class I to class II, from class II to class III, etc. Correlation coefficients may be derived for the use of the readers better acquainted with statistical methods.

It is assumed that before the above classification is made, the data included in it is homogeneous and agrees with the rules for honogeneity suggested above. If not, a further division to comply with these rules will be necessary. If the cost per quart varied according to size of dairy, each of the above classes should be subdivided somewhat as follows:

Class I, Dairies producing 2000 to 2999 pounds per cow
- A. Dairies of fewer than 20 cows
- B. Dairies of 20 to 39 cows
- C. Dairies of 40 to 59 cows.

The extent to which this sub-classification should be carried will depend, as was said above, on the nature of the particular study which is being made. In practice it is seldom possible to eliminate all but the two factors whose correlation is being considered, and it is usually necessary to permit variation in more than one factor at a time. It is best to construct the tables so that all the factors in which correlated variations could occur, will be shown in each table. Thus, it becomes possible, by cross comparison to make allowance for intercorrelation among the different factors in each class.

GENERAL FACTORS INFLUENCING THE COST OF MILK PRODUCTION

Production per cow

It is well known that the cost of producing milk, per quart or per hundred pounds, will vary inversely with the production per cow. In most investigations into the cost of producing milk, the production per cow has been made a basis for classification. In any case, it must plainly be taken into consideration, and where a comparison is made between two dairies or groups of dairies, the statistics presented should show clearly the production per cow in each dairy or class.

Size of herds

The size of herds, like production per cow, has been shown to have a strong influence on the cost of producing milk. In any study of milk production, the size of herds must be taken into consideration, and should usually be made a basis of classification. In any event, data from dairies of widely differing sizes should not be massed, and the effects of variation in size within and between different groups should carefully be watched for. The maximum and minimum sizes should be noted in summaries as well as the average or prevailing size.

Disposition of product

The disposition of product may prove a very potent factor, and should always be given some thought and mentioned in the explanatory paragraphs.

Where a study includes dairies whose products are disposed of in different ways, as for instance; market milk, butter, cheese, "A" grade milk, and "Nursery" milk, the differences in methods of dairying arising from differences in products should never be overlooked. And where any considerable difference in cost or method of management is found as such a result, the dairies should be classified according to their products.

Value per cow

The man who collects the data should have had enough experience in dairying, and should be sufficiently well acquainted with the value of cattle in the neighborhood where he is working, to be able with the aid of the farmer to place a fair valuation on the cows in each dairy.

Cows recently purchased should, of course, be valued at the price paid. Others should be valued at what they would sell for as dairy cows in that neighborhood.

In placing a valuation on the cows, their productive capacity and the likelihood of their being profitable milk producers, should be the paramount consideration. It has even been suggested[2]

[2] Bureau of Statistics, U. S. Dept. of Agriculture, Bulletin 88.

that the profit from the cows' product be capitalized as a method of evaluation. Under this method, a cow that caused a loss to her owner might be considered a liability instead of an asset. While it may be said that there is a sort of justice in this suggestion it must be remembered that even though a cow may have no value as a milk producer, she will still sell for something for beef. And there is capital tied up in her whether it be a profitable investment or not.

Pure bred cows should be valued, for the purpose of the ordinary cost of production study, at the same rate as grade cows of similar conformation and productive capacity. If a cow has a value, *inherent in the fact of her registry, above that properly pertaining to her as a milk producer*, this may properly be left out of account in such a study. In the first place, such a value does not bear any relationship to her efficiency as a milk producer, and in the second place, it may be assumed that she will impart this value to her offspring. The higher value of her calves will offset the interest on the higher value of the cow.

OVERHEAD EXPENSES

Overhead expenses are those which continue regardless of the amount of milk produced in a dairy, or of the intensity of the business. A fundamental distinction must be drawn between these expenses and those which may ordinarily be expected to increase with the amount of milk produced, and with the extent to which the dairy is forced toward its maximum limit of production.

This list will include the following factors of cost, which are of sufficient importance to merit their being discussed at some length:

1. Interest on value of cow.
2. Depreciation on cow.
3. Maintenance of buildings.
4. Maintenance of equipment.
5. Bull service.
6. Cost of bedding.
7. Miscellaneous overhead.

Interest on value of cow

The farmer may properly be allowed interest on the capital he has tied up in his dairy, whether in cows or equipment. If he had no capital of his own, he could not conduct nor begin his business until he had obtained it, either from his own savings or in the form of a loan from someone else. And a man who has capital cannot be expected to put it in a dairy unless it can be expected to return him as high a rate of interest there as if he bought sound securities, or put it in some other business. The rate of interest allowed the farmer on his investment in his farm or dairy should be the same as that at which he could borrow it, which would usually be at the rate of short time loans in the neighborhood.

Interest on the investment in cows constitutes more nearly an overhead charge than a secondary or current one, for the investment in cows is subject to no variation whether the cows be forced to their maximum production or not. Interest on the feed kept on hand, is however, more nearly a variable charge, for the amount of feed kept may be expected to vary with the amount of milk produced and the extent to which the dairy is forced.

Depreciation on cows

This is a much argued point, and several methods have been used in attempts to determine it. There have been two principal methods:

1. Depreciation on the individual cow. This is a theoretical method, whereby the difference between the value of the cow when she enters the herd, and that at death or sale is distributed over her period of usefulness—real or estimated. The weakness of this method, as it has been used, lies in the fact that the cow's period of usefulness was usually estimated rather than real. There has been a wide diversity of opinion as to what the usual period is. The usual estimates vary from five to eight years. Also there is a tendency to try to predict what a cow will probably be worth at a given age, rather than to base the final price on that of the cows actually disposed of.

Depreciation will vary with the original value of the cow, and inversely with the length of her life, but it must be remembered that she may not spend all her life in the same dairy. She may be sold at just the time she has reached her greatest value. Thus, some cows will not depreciate but will appreciate in value. This is an important fact, which such a formula as would be derived from the above method fails to take into account. The writer knows of some dairies where the practice is to sell cows before they have depreciated to any considerable degree, and a charge based on the above method would be altogether wrong in them. On the other hand, a formula which would represent an appreciation until the cow is say five years old, and then a graduated depreciation might provide the most accurate system of all.

2. Depreciation on the herd. By this method, the actual depreciation on a herd for a given year, is estimated as closely as possible, and this amount is divided by the number of cows to get the average depreciation per cow. This amount is arrived at as follows: to the inventory at the beginning of the year, add the price of young stock or new cows entering the herd. From this sum is subtracted the inventory at the end of the year plus the price received for cows sold. This is then divided by the number of cows in the dairy.

It has been objected, that in the case of cows which are sold during the year, an element of profit on such sales will enter into the sum arrived at by this method, as depreciation, and that this will to some extent, invalidate the charge. However, it should be remembered that, in the case of cows purchased, it may just as well be assumed that their price likewise contained an element of profit to the seller, which will offset to the buyer the profit he makes on the cows he sells. At any rate, this difference, in most cases will be so small that the refinement of method necessary to dispose of it, will scarcely be worth while. Depreciation may be computed accurately only after the cow has passed out of the herd, but it is necessary to make some sort of allowance for it each year, and it is believed the herd method is sufficiently accurate for all ordinary purposes.

Allowance should be made for fluctuation in market price, from year to year, as this is not true depreciation or appreciation.

Maintenance of buildings

This factor comprises several subfactors which will be discussed separately.

1. Interest on investment. The buildings should be inventoried at the beginning and end of the year. Interest should be charged at the rate the farmer would have to pay if he borrowed the capital invested in the buildings. It is a better business policy to charge interest on the inventory at the beginning than at the end of the year. The principal effect of charging the interest on the basis of the depreciated value at the end of the year, will be to lower the credit the farmer receives for interest. However, this amount of reduction will not be uniform, since buildings differ in their durability. A fairer and more uniform rate of interest will be allowed if it is always charged on the undepreciated value.

2. Depreciation on buildings. This varies with the type of the building, but usually runs at from 2 to 4 per cent. It is found by subtracting the inventory at the end of the year from that at the beginning of the year.

3. Repairs. A distinction must be kept in mind between repairs, which are of the nature of current expenses and have the effect of making the inventory at the end of the year equal to that at its beginning; and additions which are to be added to the capital invested, and not the current expense account.

4. Taxes. The buildings are debited with the actual tax, that is, with the tax paid, at the local rate on the assessed value of the buildings.

5. Insurance, is charged at the rate actually paid.

It is not proper to charge for maintenance of buildings, a rate which would cover maintenance of a set of "theoretical buildings," that is, on buildings costing what it is estimated should be sufficient to provide shelter for the herd. But in the case of buildings only part of which are used by the dairy, the charge for maintenance should be made only on that part of the building which is actually used by the dairy.

Maintenance of equipment

This factor includes:

1. Interest on the capital invested in the equipment.
2. Repairs.
3. Depreciation. This factor is generally pretty high, and may run as high as 25 per cent. It may be defined as a charge which is sufficient to maintain the equipment from year to year in equally good repair and to replace articles of equipment as they wear out. Additions to equipment, like additions to buildings, should be added to the capital invested and not to the depreciation account.
4. Tax and insurance on equipment.

These items in the ordinary dairy are so small as to be negligible. However, when an expensive equipment is used, they may assume very noteworthy proportions and must therefore, be taken into consideration.

Cost of bull service

Several different methods have been used in efforts to determine the cost of bull service. None of them have been completely accurate nor eminently satisfactory.

A close approximation to the true cost of bull service is generally the best that can be hoped for. The difficulty of ascertaining the cost of keeping any one of a herd of animals will be readily appreciated when it is borne in mind that farm accounts are poor at best, and the bull is fed out of the same feed bin as the rest of the herd, and cared for by the same men, without any careful account being kept of his feed or care.

The cost of bull service will comprise:

1. Interest on the value of the bull as a herd sire.
2. Depreciation, figured out by practically the same method as for cows, but the period of usefulness will not be likely to be the same as for the cows.
3. Feed.
   a. Grain, usually somewhat less than for a cow.
   b. Forage, usually slightly more than for a cow.

4. Taxes on his assessed value at the local rate.
5. Expense for shelter.
6. Labor. The bull generally requires more labor than a cow in feeding and caring for him, but this will be offset by the time required to milk the cow.
7. The bull must be charged with his share of the unassignable overhead.

The total cost of keeping the bull is pro-rated among the cows. Only the bulls actually in service should be considered in a study of the cost of milk production. The young bulls, not yet in service, cannot be considered as belonging to the dairy herd.

The device sometimes used, of assuming the cost of bull service to balance with the value of calves at birth, is entirely invalid. The cost per cow for bull service will depend mostly, on the number of cows for which a bull is kept.

Bedding

The cost of bedding is computed in much the same manner as the cost of forage. It is comparatively easy to compute the value of straw used for bedding, because it generally has a market price. The cost of some other materials used for bedding, such as shredded corn fodder, and refuse hay, is not so easy to find, and if their cost cannot be found it becomes necessary to compute their value on the basis of the amount of straw or the usual bedding which they replace.

In the case of some roughages, where the cows eat only a part, and where the rest is used as bedding, it will be necessary to make an approximation of the portion finally used as bedding rather than as forage. This amount is to be charged to the account of bedding rather than that of forage, and at bedding price.

A number of investigations have lumped the expenses for bedding and for forage. A distinction should be made between forage, which, used as feed, stands in some correlation to the amount of milk produced, and bedding which is necessary to the comfort of the cows, and to the production of good manure, whether much or little milk be produced.

Miscellaneous overhead

This will include those minor expenses, which do not vary with production, and which are not of sufficient size to merit consideration as separate factors.

1. Insurance on life of cow.

This factor will be practically constant in all but very exceptional dairies, and may be computed from the mortality tables of live stock insurance companies.

2. Veterinary fees, medicine, disinfectants, etc.
3. Salt, stockfoods, etc.
4. Tax on the assessed value of the cows at the local rate.
5. Water and light.
6. Ice.
7. Cow testing association fees, registration fees, etc.
8. Incidental expenses, as a portion of telephone bills, cost of hiring labor, etc.

VARIABLE EXPENSES

Forage

Forage should be charged to the dairy at farm value when it has thoroughly cured in the mow, that is, at the earliest time at which it could be sold.

In the case of roughage which has no market value to serve as a basis for farm valuation, it may be necessary to compute value as compared to some roughage which does have a market value. Corn fodder may come under this head, but even it has some sort of a market price, as may be found out by visiting a public sale of a farmer's effects.

If interest is to be allowed on the amount of feed kept on hand, this must be charged to the dairy at the farm price at the time the feed is stored, in order to be consistent. The capital on which the farmer may be allowed interest, is that which he tied up by deciding to keep his own feed after the earliest date at which he could have sold it.

The justice of valuing farm crops at the market price (minus cost of delivery) will be appreciated when it is considered that,

in the case of crops grown to sell, the farmer becomes a sort of speculator in his own produce if he does not sell it at the earliest opportunity. Prices may rise later, or they may fall. At any rate, the produce will shrink, and lose weight, which will partly offset any rise in price to come later. The farmer must also provide for, or bear himself, the insurance on loss by fire, storm, and depredation by vermin. Hence, if he keeps his crops, it is at his own risk and his own expense.

On the other hand, if feed is charged to the dairy at the market price at the time it is fed, no interest on its value, nor expense for storage can consistently be charged.

Grain

Grain grown on the farm, should be charged at the farm value at the time of storage, as for forage. Farm value is the market price at the time it is threshed or put in the barn, minus the cost of selling and transporting it to the market.

Concentrates which are purchased, are charged at the price paid, plus the cost of transporation to the farm. In some dairies, feeds may be hauled by the team returning from the creamery or milk station. Here it is not fair to charge the whole expense of hauling either to the feed account or to that of hauling milk, but an allotment of a part of the expense must be made to each.

Silage

Silage should be charged to the dairy at its cost as nearly as that can be found. Since silage is ordinarily grown for dairy feed only, and has no market value in the usual sense, it cannot reasonably be charged at any estimated value. Growing silage must be considered as an adjunct of the dairy business, hence it should be charged at cost.

The same applies to soilage and root crops which are grown solely for dairy feed and not for sale.

Pasturage

Many bulletins have taken what seemed to be the prevailing rate of pasture hire in the neighborhood as the amount to be

charged for this factor. This is hardly a valid method of procedure because, in a bona fide farming neighborhood, there is generally very little "pasturing out," and the rate so computed may not be representative of the actual cost.

The actual cost of pasture may be found by adding together the following factors:

1. Interest on the value of the pastured land, at the prevailing rate in the neighborhood.
2. The cost of keeping up the fences of the pastures.
3. Cost of reseeding, mowing, etc.
4. Fertilizers and top dressing applied to the pastures.
5. Taxes on the pasture lands at the local rate.

Labor

Many and varied methods have been employed in computing the cost of labor, but the simplest and the one which measures individual differences most accurately will prove the most reliable and satisfactory in the long run, and the most dependable in making comparison between different dairies or groups of dairies.

The actual cost is to be found by adding up cash wages, and value of board, and other perquisites which the laborer receives. This sum is divided by the number of hours the man worked during the month or week, as the case might be, to find the cost per hour. No greater period of time than the month should be used in computing the cost per hour of labor, because otherwise an error would enter in from the seasonal variation in wages.

Horse labor

The cost of keeping a horse on a given farm for a year, must serve as the basis for finding the cost of horse labor per hour, the cost per year being divided by the number of hours of labor the horse performed during the year to find the cost per hour.

It is necessary to use the cost of keeping the horse a year instead of a month, as in the case of man labor, because of the wide seasonal variation in the number of hours of horse labor performed per day on the average farm. If it is necessary to keep the horse during the winter, when he does not work, in order to

get his services during the summer, it is only fair that the enterprises on which the horse works during the summer should bear a share of the expense of keeping him during the winter.

Cost of hauling milk

In a study of this sort, there are two possible alternatives.

1. To find the cost of producing milk on the farm and leave out of account the cost of hauling, or in other words, to find a farm cost.

2. To find the cost of the milk as delivered at the creamery or milk station.

Since the latter is the actual cost to the farmer, and he seldom sells his milk on the farm but is required to deliver it to some point at a distance, it will usually be the one used. The following method of computation may be used;

1. Find the actual number of hours spent in hauling the milk and in returning to the farm, as nearly as possible.

2. Multiply this number of hours by the cost per hour of the labor of the man who drove the milk wagon.

3. Multiply the number of hours by the cost per hour of the horses labor.

4. Add a reasonable amount for depreciation on the value of the wagon and harness.

It may be desired to know the cost of hauling a can of given capacity, per mile. This may be easily found by multiplying the number of cans of milk hauled by the number of miles to the milk station, and dividing by this product the total cost for hauling.

Incidentals

This includes those items which vary with the amount of milk produced, but are not of sufficient size to merit individual treatment. Such items are:

1. Feed grinding.

2. Storage of feed.

3. Interest on funds usually kept on hand to cover operating expenses.

4. Interest on the capital tied up in stored feeds, including both those bought and those grown on the farm. If this interest is allowed the feed grown on the farm must be charged to the dairy at the farm price at the time it is put in the barn. And if this charge is not allowed the feed will be charged at the price when it is fed. The price at the time the feed is fed, will include, theoretically at least, a charge for storage, and for interest. The charge for storage, if it is made, may be either incorporated in the price charged the dairy for the feed or, perhaps better, made separately, while the interest is added to the general charge for interest.

CREDITS

Credits for milk

Credit for milk will comprise the annual income from milk delivered at the milk station or creamery. If it is desired to compare prices received in different dairies, it will be necessary to reduce the prices to some comparable status. Some dairies deliver their milk to creameries, and so have no freight to pay. Others ship to distant dealers, and the price they receive must be reduced by the amount of the freight before it can be compared to that received by the other class.

All milk used at the farm for home consumption, and for raising calves, after the earliest time at which the calves could be weaned, comes under this account. Skim milk in dairies where the milk is skimmed on the farm, and only the cream or butter sold as a direct product of the dairy, is also to be included under the heading of credit for milk. This skim milk is usually marketed indirectly by feeding it to hogs, and is thus a considerable source of income to the farm.

Credit for calf

The dairy should be credited with the value of the calf at three days of age, the time at which it could be separated from the cow.

For the purposes of the ordinary cost of production study, calves of similar size, conformation and vigor should be valued

at the same rates, whether they are grades or pure breds. To introduce the factor of additional value inherent in the fact of registry would greatly complicate the study, and for the purposes of such studies, this peculiarity of pure bred calves may be considered as a return on the corresponding value of their dams. This device of valuing pure bred calves at the same rate as grades, also conforms to the system given above for valuing the cows.

Credit for manure

Manure should be valued at a rate which would enable the farmer to buy equivalent amounts of other fertilizers containing the same elements of plant food. Allowance should also be made for the value of manure as improving the physical and biological properties of the soil. It will usually be necessary to adopt a uniform rate per ton. It will seldom be possible to go into such refinements of method as to place separate valuations on the manure on different farms, although such differences are known to exist as a result of different methods of handling manure, excessive leaching on some farms, etc.

The rate adopted per ton should be determined by the usual or average condition of manure in the neighborhood, when it is hauled to the fields.

Other credits

In most dairies there will be a few other credits than for milk, calves, and for manure. These are such as for hides, bags, etc. They are not likely to form a very important factor but they should not be lost sight of.

In the dairy business, the milk is the foremost aim of the business, and the other products are merely incidental to the production of milk. Hence, in finding the cost of the milk, the sum of all other credits are first subtracted from the total cost of production, and the remaining, or net cost is taken to be the cost of the milk.

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